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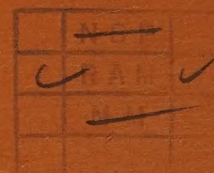
THE
REGIONAL RESEARCH CENTRE
of the British Caribbean

AT

THE UNIVERSITY COLLEGE OF THE WEST INDIES
IMPERIAL COLLEGE OF TROPICAL AGRICULTURE
TRINIDAD, W.I.

A REPORT ON
CACAO RESEARCH
1959-1960

June, 1961



The Regional Research Centre for the British territories in the Caribbean came into being on 1st September, 1955. The nucleus of the Centre has been formed from the staffs of the three research schemes for bananas, cocoa and soils which were wound up on 31st August.

The Cocoa Research Scheme made on 4th December, 1947, provided for the maintenance of type collections and for research on all problems connected with the cultivation, genetical improvement and health of the cocoa tree and of its fruit. In the agreed five-year research programme, effective from 1st September, 1956, it is stated that "Research on cacao has two main practical objectives: (a) the elaboration of techniques for removing factors that limit optimal yield and (b) evolution of high-yielding trees bearing beans of desirable flavour." It is recognised that the research of the Cocoa Section of the R.R.C. is not confined to Caribbean problems but, as far as local circumstances allow, is world-wide in its range.

The Rt. Hon. Harold Macmillan inspecting the cocoa research exhibit during his visit to the Imperial College of Tropical Agriculture. (Photo Chan's Photographers Ltd.)



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1959-1960



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THE REGIONAL RESEARCH CENTRE
of the British Caribbean
at
THE UNIVERSITY COLLEGE OF THE WEST INDIES
IMPERIAL COLLEGE OF TROPICAL AGRICULTURE

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of the
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Introduction

THIS Report is the first to appear since the merger, on the 1st August, 1960, of the Imperial College of Tropical Agriculture with the University College of the West Indies in Jamaica. The Imperial College has become the Faculty of Agriculture of the University College and in consequence the Regional Research Centre is now administered by the University.

Other faculties, of which the first is engineering, will be developed at St. Augustine. One result of this is the loss of some experimental land at St. Augustine for the building

programme but as the greater part of the cacao field work has been done at River Estate and, more recently, Las Hermanas the consequences of this are not very serious.

Shortly before the merger Dr. Herklots resigned as Principal and Director of Research. He had been largely responsible for the amalgamation of the old Cocoa Research Scheme with other research schemes to institute the Regional Research Centre in 1955. The policy as laid down then continues to serve as the basis for the research programme.

A review of progeny trial performance, 1956-60

by

B. G. D. Bartley

AMONG the first steps in the development of the cacao breeding programme was the comparison of the progeny of crosses among the best ICS clones and crosses of the Witches' Broom resistant clones, SCA 6 and SCA 12, with some ICS clones. The progeny trials provide populations for the selection of individuals of superior merit. They also provide information on the breeding behaviour of the parents and the use of bi-clonal hybrids on a commercial scale. The data obtained during the first years of bearing from the first progeny trials were discussed by Bartley and Cope (1957) and Bartley (1958). The present paper is a summary of the results obtained up to the end of the 1959-60 crop. The progeny trials are discussed according to the age of the trials.

Trial No. 1

The trial compares three clones ICS 1, ICS 6 and ICS 8 and the progeny of the three combinations of these clones. With the exception of ICS 6 $\times$ ICS 8, which was planted in 1951, the trial was planted in 1950 at a spacing of 12 ft. $\times$ 12 ft. Shade is provided by old *Erythrina* trees and apart from a dressing of sawdust and stable manure in 1959 no other treatments have been applied. A plot comprises 25 trees and the varieties are planted in a randomized block layout with four replications. The varieties have exhibited considerable variation with respect to pod infection by Witches' Broom. The extent of losses due to Witches' Broom are given in Table I. Losses on ICS 8 were considerably higher than those of the other varieties. In order to obtain a better estimate of the potential yielding ability of the varieties individual tree yields were adjusted to compensate for diseased pods.

The yields from healthy pods and estimated potential yields are presented in Table I. Up to 1958-59 yields were relatively low and declined. The 1959-60 crop showed a large improvement with some varieties increasing in yield by about 300% over the previous year. Although ICS 8 had the highest estimated yield in the first three years it had a relatively low increase in 1959-60 and gave a lower average yield than ICS 6 for the four years.

The seedling progeny consistently gave lower yields than the clones. ICS 1 $\times$ ICS 6 gave the lowest average yield and was particularly poor in 1958-59 when the yields from two plots were negligible. The increase in yield for 1959-60 was as large in the seedlings as in the cuttings. Although ICS 6 $\times$ ICS 8 was planted one year later than the other two crosses it outyielded them from the sixth year. The difference between ICS 1 and ICS 8 and the yields of the crosses in 1959-60 was not significant. The relationship between the yields of ICS 1 and the progeny of ICS 6 $\times$ ICS 8 is shown in Fig. 1.

Trial No. 2

This trial was laid down to compare the progeny of ICS 1, ICS 6 and ICS 60 as female parents crossed with the Witches' Broom resistant clones SCA 6 and SCA 12. A complete description of this trial, which was planted in 1951, and the results obtained up to 1956-57 were given by Bartley (1958). Since 1957 the field has received an annual application of NPK fertiliser at the rate of one pound per tree. The cuttings which were interplanted with the seedlings in this trial appeared to be of little value and were cut out in two stages, half of the plants being removed in 1959 and the remainder in 1960. At the

TABLE I

Yields (lbs. dry cocoa per acre) of healthy pods (A), potential yields (B), and percentage pod infection by Witches' Broom in Trial No. 1 for 1956-1960

Variety	Yields (lbs. per acre)								Witches' Broom pods			
	1956-57		1957-58		1958-59		1959-60		(per cent of total crop)			
	A	B	A	B	A	B	A	B	1956-57	1957-58	1958-59	1959-60
ICS 1	680	740	610	665	600	650	1,165	1,475	6.2	6.1	6.8	20.4
ICS 6	710	760	675	725	520	540	1,530	2,095	6.7	7.0	5.2	26.7
ICS 8	660	875	620	795	615	780	740	1,440	20.5	20.3	19.5	48.1
ICS 1 $\times$ ICS 6	300	330	400	430	240	255	920	1,185	7.6	6.0	3.7	20.0
ICS 1 $\times$ ICS 8	520	575	370	415	360	380	825	1,040	5.0	7.3	4.4	19.7
ICS 6 $\times$ ICS 8	470	500	535	605	380	405	965	1,375	7.1	10.7	4.2	28.7
S.E. difference (excluding ICS 1 $\times$ ICS 6)		132		91		82		205				

present time the trees are spaced at 7 ft. $\times$ 14 ft. at a density of 444 trees per acre.

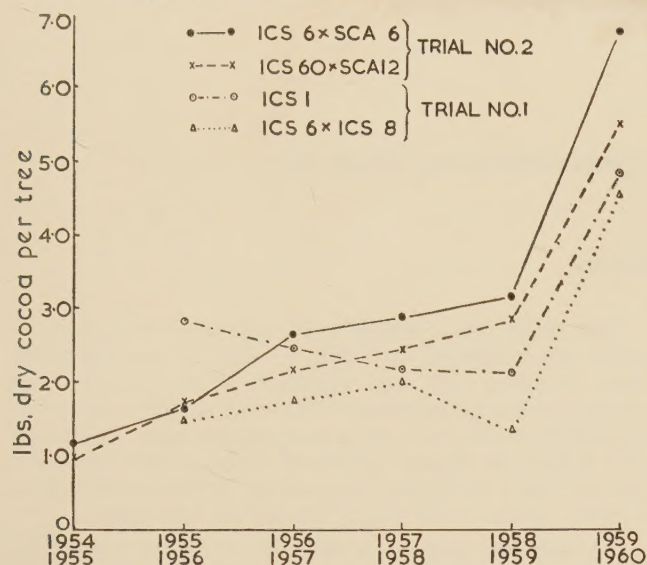


FIGURE 1

Yields (lbs. dry cocoa per tree) for ICS 1 and ICS 6 $\times$ ICS 8 (Trial No. 1) and for ICS 6 $\times$ SCA 6 and ICS 60 $\times$ SCA 12 (Trial No. 2) 1954-1960.

In certain parts of the field considerable damage to pods by squirrels occurred. This damage was localised and individual tree yields were adjusted to account for the pod losses.

Yields for the three years from 1957-60 are presented in Table II. In view of the change of spacing due to the removal of the cuttings it was more satisfactory to present yields in terms of production per tree rather than on an acre basis. Consistent increases have been obtained each year except in 1959-60 when yields were double those of the previous year. The crosses maintained the same ranking and the same relative trends in yield over the years and Fig. 1 shows the relation between two of the crosses in the trial.

TABLE II

Yield (lbs. dry cocoa per tree) for six crosses in Trial No. 2 for 1957-1960

Cross	Yield (lbs. per tree)		
	1957-58	1958-59	1959-60
ICS 1 $\times$ SCA 6 ...	2.19	2.52	5.65
ICS 1 $\times$ SCA 12 ...	2.56	2.65	5.42
ICS 6 $\times$ SCA 6 ...	2.89	3.15	6.80
ICS 6 $\times$ SCA 12 ...	2.48	2.47	5.42
ICS 60 $\times$ SCA 6 ...	2.08	2.01	5.30
ICS 60 $\times$ SCA 12 ...	2.45	2.86	5.55

The progeny of ICS 6 $\times$ SCA 6 have consistently given the highest yields. In general, crosses involving SCA 12 have outyielded those of SCA 6, except ICS 6 $\times$ SCA 12 where some trees are a year younger. The progeny of the Criollo clone ICS 60 gave lower yields than the progeny of ICS 1 and ICS 6. The large increase in yield in the 8th year (1959-60) could not be attributed solely to the reduction in tree density as a similar increase was obtained in Trial No. 1 in the same year (Fig. 1).

The proportion of pods infected by Witches' Broom was less than 1% in 1959-60 and no change in the relative susceptibility of the crosses was observed.

Trial No. 3

The treatments in this progeny trial include 32 crosses among twelve ICS clones, and these crosses are compared to their parents as cuttings. The trial was planted in 1953 and 1954 at a spacing of 10 ft. under existing *Erythrina* trees. No fertiliser has been applied in this field. Each cross is represented by 100 progeny in 4 plots which are completely randomized. The parents are each represented by 30 trees in 6 plots of 5 trees which are also randomized within rows throughout the field, there being one row of cuttings to 10 rows of seedlings.

The progeny of some crosses, such as ICS 1 $\times$ ICS 98, were planted over a period of two years and the yields from these crosses show much variation. Other crosses, like ICS 8 $\times$ ICS 98, were planted in 1954 and are a year younger than most of the trees in the field. Most crosses have suffered damage through falling shade trees and *Ceratostomella-Xyleborus* infestation. The latter has particularly affected trees of Criollo parentage and these have been omitted from the records.

The yields of the eleven best crosses and their parents for the years 1958-60 are given in Table III. The best parents, ICS 39, ICS 40 and ICS 60, gave yields of about 1,400-1,600 lb. dry cocoa per acre in 1959-60. If these clones are not considered it will be seen that the yields of the best crosses of Trinitario origin are comparable to those of their parents. In fact, many of the best combinations are those between the lower yielding parents. While ICS 98 gave the lowest yields it occurred in five of the eleven best crosses.

Trial No. 4

This trial was planted in Field 19, also in 1953. The object was to compare four clones in four treatments (a) as clones, (b) open-pollinated progeny, (c) crosses with ICS 1 and (d) crosses with ICS 6. One of the aims of this trial was to compare the merits of ICS 1 and ICS 6 as top-cross parents. Open-pollinated progeny were included to compare the performance of these progeny with that of the offspring of crosses among known parents. This comparison was made owing to the confusion caused by the suggestion (Jolly, 1956) that offspring from uncontrolled pollination provided a suitable measure of the usefulness of seedling plants.

The four parent clones selected were ICS 39, ICS 89, ICS 95 and ICS 98, providing a comparison of different sources of genes and including a range of performance and adaptability. The 16 "treatments" were planted in a randomized block design with 4 replications and 20 trees per plot. The spacing was 8 ft. $\times$ 8 ft. and shade was provided by existing *Erythrina* trees. Two crosses, ICS 95 $\times$ ICS 6 and ICS 98 $\times$ ICS 6, could not be planted until 1954, and their yields are considerably lower than those of the other treatments making it difficult to draw conclusions as to the relative combining abilities of ICS 1 and ICS 6. The close spacing has had an adverse effect on yield particularly because of the variable nature of the experiment. *Ceratostomella* infection has been severe in the Criollo trees and consequently has reduced the validity of some of the comparisons.

TABLE III

Yields (lbs. dry cocoa/acre) from the 11 best crosses and their parents as cuttings in Trial No. 3 for 1958-59 and 1959-60

Cross	Yield (lbs. per acre)		Clone	Yield (lbs. per acre)	
	1958-59	1959-60		1958-59	1959-60
ICS 89 × ICS 8	645	875	ICS 1	1,110	985
ICS 1 × ICS 98	630	840			
ICS 1 × ICS 89	595	930			
ICS 40 × ICS 98	590	675			
ICS 89 × ICS 95	585	700			
ICS 60 × ICS 98	520	610			
ICS 39 × ICS 98	490	725			
ICS 39 × ICS 8	375	700			
ICS 1 × ICS 40	470	680			
ICS 40 × ICS 8	450	675			
ICS 8 × ICS 98	430	635			
			ICS 6	1,110	825
			ICS 8	660	590
			ICS 39	1,390	1,450
			ICS 40	1,385	1,620
			ICS 60	1,200	1,380
			ICS 89	300	450
			ICS 95	900	980
			ICS 98		360

Differences between the treatments became apparent at an early stage. Their performances for the 1959-60 crop year, presented in Table IV, show the relative positions of the clones and their progeny. In this trial also the progeny of ICS 39 yielded considerably less than the clone itself, and the yield of the open-pollinated progeny was not different from that of the crosses. The situation with ICS 95 progeny was similar except that the crosses appeared to be capable of giving higher yields than the open-pollinated progeny. The progeny yields from ICS 95 were lower than those of ICS 89 and ICS 98 which were the lowest yielding parents and whose crossed

on the testing of a large number of crosses among different groups of parents. From these crosses the parents with the highest general combining ability may be selected, and the best combinations among these parents determined by further testing. This system of breeding forms the basis for the present cacao improvement programme.

In general its yield performance is no criterion of a selection's combining ability. Trees of Criollo origin, although of high yield potential themselves, are inferior as parents and do not combine well with other groups of cacao. If to this is added the marked susceptibility to diseases of the Criollos it would appear that they will play

TABLE IV

Yields of cuttings, open-pollinated and top-crossed progeny of 4 clones in Trial No. 4 during the 6th year (1959-60)

Parent	Yield (lbs. dry cocoa per acre)			
	Cuttings	O.P. Progeny	× ICS 1	× ICS 6
ICS 39	1,375	600	575	615
ICS 89	410	530	830	620
ICS 95	860	425	520	315*
ICS 98	660	625	825	455*

* 5 years of age.

progeny were superior (in the ICS 1 crosses) to the open-pollinated offspring.

ICS 89 is considered to be a clone of great merit but has the characteristic of late bearing. The increased yields of the progeny may indicate that earliness of bearing is a dominant character or that early bearing is due to factors determining growth rates which are independent of yielding ability. The data show no definite differences in combining ability for ICS 1 and ICS 6. Two crosses, ICS 89 × ICS 1 and ICS 98 × ICS 1, yielded as well as the ICS 95 cuttings.

Conclusions

A wide variation in yield performance exists both in parent clones and in the offspring of bi-clonal crosses. A considerable potential appears to exist in the latter type of variety and efficient use of such a method will depend

a minor role in the programme of breeding for performance.

Yields of bi-clonal crosses of Trinitario parents are more encouraging than previously reported. With proper use of the material available, improved breeding techniques and control of subsidiary characters, it should be possible to obtain bi-clonal crosses equal in performance to the best Trinitario clones.

However, the use of Amazon material seems to offer the best possibilities of yield improvement. Such material has not been fully exploited in Trinidad, mainly on account of the small seeds of the selections. The increasing yields of the Trinitario-Scavina hybrids indicate the possibilities of combining Trinitario and Amazon germplasm. The highest yields from Trial No. 2 at the present spacing of 14 ft. × 7 ft. are about 3,000 lb. dry cocoa per acre. The main disadvantage of the Trinitario-Scavina hybrids is the

small seed size. In order to combine the yield potential of some of the Amazon selections with large seed size a study of seed size inheritance is necessary.

The results obtained from the four trials described indicate that the ICS clones which show most promise as parents are ICS 8, ICS 89, ICS 98, ICS 6 and ICS 1. This is a limited selection of parents and it will be necessary to enlarge the collection of germplasm to provide a wider basis for the testing of parents.

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Early yields of some clones introduced into Trinidad

by

D. B. Murray

FOLLOWING the lead of Trinidad, selection programmes have been undertaken in a number of cacao-growing countries. These have given rise to a number of clones many of which have been introduced into Trinidad. It was of some importance to investigate the performance of these under Trinidad conditions and to compare their yields with those of the better ICS clones.

When sufficient planting material had been bulked up, two field trials were planted at River Estate in 1955. The origins of the clones were as follows:—

GS clones	Grenada
DS	„
UF	„
CC	„
SIC	„
E	„
	Dominica
	United Fruit Company, Costa Rica
	Turrialba, Costa Rica
	Belem, Brazil
	San Juan Estate, Trinidad

The first trial 1955 (a) carried 19 introductions and two ICS clones. The layout was a balanced incomplete-block design in which each clone was replicated five times. Plots of only four trees were used to economise on land, especially as the trial would constitute only a preliminary screening. A spacing of 12 ft. × 9 ft. was adopted under a light overhead shade of *Erythrina*. Guard rows of ICS 60, which is very susceptible to Witches' broom, were planted between plots as a source of infection.

The trees came into bearing in the 1957-58 season and the yields for the first three crops follow. The table includes the weight of wet beans per pod and Witches' broom rating. For this latter the plants were scored for vegetative attack in relation to ICS 1, mild - 1, up to ICS 39, severe - 4.

The second trial 1955 (b) planted in the same field contained seven clones in five randomised blocks. Plot size and other factors were similar to those of the previous experiment. This trial however was not planted until October, *i.e.* late in the rainy season as compared with June planting for the first trial. This four months' delay in planting produced a very marked effect, setting back the plants by a year in regard to yields. This result emphasises the importance of early planting in the field in relation to season. This trial accordingly did not come into bearing until 1958-59 and the yields for the two-year period follow.

These early yield figures, though subject to high variability, give indications of the potential of these introductions under Trinidad conditions. All the UF clones show considerable promise, as do the best of the Grenada selections. In Grenada, the two outstanding local selections are GS 29 and GS 36 and it will be noted that here these same two, with GS 46, head the Grenada list. None

Introductions Trial 1955 (a)
Yields in lbs. dry cocoa/acre

Clone	Age			Total	Wet weight beans per pod (gms.)	W.B. score
	2 years	3 years	4 years			
ICS 39	310	430	1,090	1,830	144	4
UF 168	200	420	1,100	1,720	133	4
UF 654	260	350	840	1,450	119	4
UF 667	200	300	940	1,440	130	4
GS 29	190	400	820	1,410	126	2
UF 650	170	280	820	1,270	132	4
UF 221	180	320	590	1,090	132	4
GS 36	120	320	640	1,080	125	3
GS 19	160	400	430	990	103	2
ICS 1	90	280	530	900	123	1
GS 46	130	250	500	880	120	3
GS 53	120	250	400	770	124	3
GS 5	100	240	320	650	135	2
GS 65	50	160	360	570	143	3
SIC 2	100	250	180	540	77	2
DS 9	70	140	290	500	110	2
CC 4	200	80	170	450	99	4
GS 18	40	110	270	420	144	4
DS 1	40	90	230	360	103	3
GS 11	40	120	130	290	129	3
GS 77	40	100	80	210	99	2
L.S.D. 5%	±116	±179	±506			

of the Dominica selections, with the possible exception of DS 3, shows any promise in Trinidad; nor do SIC 2 or

Introductions Trial 1955 (b)
Yields in lbs. dry cocoa/acre

Clone	Age		Total	Wet beans per pod (gms.)	W.B. score
	3 years	4 years			
ICS 1	92	452	544	115	1
DS 3	82	346	428	121	2
GS 57	43	332	375	86	3
GS 15	10	273	283	139	3
GS 71	26	156	182	109	3
GS 78	25	151	176	133	3
E 575	14	88	102	127	1
L.S.D. 5%	—	± 128			

CC 4, and even if their yields had been higher their small pod size would rule against them. The selection E 575 from a high-yielding tree at San Juan Estate shows no promise, despite the fact that every possible precaution was taken in assessing the potential of the mother tree. This serves to emphasise the importance of always submitting to test the vegetatively propagated progeny of apparently outstanding mother trees. As with the original ICS selections a high proportion of field selections do not survive their screening trials. All clones showed varying degrees of susceptibility to Witches' broom, the UF clones being heavily attacked. No clone is better than ICS 1 in this respect.

Acknowledgment

The writer's thanks are due to Dr. F. W. Cope for assistance with the balanced incomplete-block design and statistical analysis.

The I.C.T.A. controlled environment growth rooms

by

D. B. Murray

PROBLEMS in agriculture need to be tackled from various angles and by a range of techniques. Since experimentation is the basis of all scientific research it is obvious that the scientist must know and define just what his experiment is to test. In the physical sciences, experimental work is done under carefully controlled conditions so that cause and effect can be directly related; in the biological sciences, however, control of all the possible interacting factors is extremely difficult. This is especially so for the plant growing in its natural environment in the field. Here the effects of rainfall, humidity, soil moisture, temperature, light intensity, etc., are varying all the time and not in direct relation to each other. To determine the response of the cacao tree to changes in just one factor at a time is therefore extremely difficult.

The first approach to the problem is by direct measurements in the field. For example, continuous records of temperature may be taken and at the same time measurements of the rate of growth of the tree. Using statistical methods it may then be possible to derive a correlation between the two measurements. Thus Humphries (1944) showed that flushing in cacao tended to be associated with mean day temperatures higher than 83°F. Later, Alvim (1956) found that there was a closer relationship between flushing and the difference between maximum day temperature and minimum night temperature. In this type of work, however, the correlations are never very high and it is only possible to say that some relationship exists but not to define the precise effect of a change in any one factor.

For this it is necessary to adopt the methods of the physical sciences and devise means of producing controlled experimental conditions which can be varied at will. This means growing plants under artificial lighting in rooms where the temperature and humidity can be fixed at whatever experimental level is required.

The first installation on a large scale to provide these facilities was the Phytotron at the California Institute of Technology constructed by Went (1957) at a cost of over U.S. \$400,000. The work done there stimulated great interest in the use of controlled environment for plant studies and Hudson (1957) states that in Britain, although there is nothing on the scale of the Phytotron, there are some seventy University departments and research stations with facilities for this type of work.

Studies of the response of cocoa to its environment were initiated at the Imperial College of Tropical Agriculture nearly thirty years ago when McDonald (1932) selected an area of "good" cacao and an area of "bad" cacao and tried to determine the significance of the environmental factors in producing the differences between the plots.

He measured some seven environmental factors and established certain general relationships but for the reasons already given no precise interrelations could be sorted out. At the London Cocoa Conference in 1955, Murray (1955) in discussing the climatic requirements of cacao first drew attention to the necessity of using controlled environment growth rooms for the study of the growth of cacao and in 1960 funds were made available for the building of growth rooms with the necessary facilities.

General description

There is little detailed information on the construction and operation of growth rooms and although such information as could be obtained was sought, the general design and equipment for the present layout has had to be developed on the spot. Among other problems was the fact that little work has been done in growth rooms on tree crops and also as far as the author is aware this is the first installation in the tropics.

The cost of growth rooms increases with the degree of control required and the complexity of the arrangements for securing it. For this reason and because technical help to deal with problems of the equipment was not readily available it was necessary to design as simple and foolproof an arrangement as possible. It was decided to build three identical rooms for two reasons. Obviously two rooms comparing a factor at two levels is the minimum installation. With one extra room it is possible to compare three levels or two factors at two levels. Furthermore in the case of a breakdown in equipment one room as reserve will ensure that months of experimental work are not entirely lost. The building is shown in Fig. 1.



FIGURE 1

The growth rooms. Note access to ceiling at right.

Each room is 10 ft. $\times$ 10 ft. $\times$ 9 ft. and the size was planned so that a room would hold four cacao plants of an age and size to be flowering and fruiting. Construction is of hollow clay blocks, cement plastered, and no additional insulation is provided. A concrete floor, sloped for drainage, is painted white like the walls to give maximum light reflection. The ceiling is of asbestos sheets also painted white.

A covered passage-way surrounds the three rooms to keep the sun from striking the walls and the various controls are sited in the back passage. Special consideration was given to the roof and ceiling area. As this contains the auxiliary lighting equipment it must be kept as cool as possible and the roof was therefore designed to allow the free passage of cooling air from the direction of the prevailing winds. Access to the ceiling area is by an outside stairway.

Temperature control

Facilities are available for the maintenance of temperatures both lower and higher than ambient. A commercial 2 h.p. air conditioner provides cooling and this is controlled by a remote mercury-in-metal thermostat operating through a relay. With this, temperatures down to 68-70°F. can be achieved and maintained with a constancy of $\pm 1\frac{1}{2}$ °F. When high temperatures are needed the compressor on the air conditioner is switched off and the air flow directed over three 1,300-watt electric elements. These can maintain temperatures in the room of over 95°F. Control by a bimetallic thermostat was not satisfactory and the heaters are now controlled by the same system as the air conditioners. Fig. 2 shows the arrangement of the heaters.

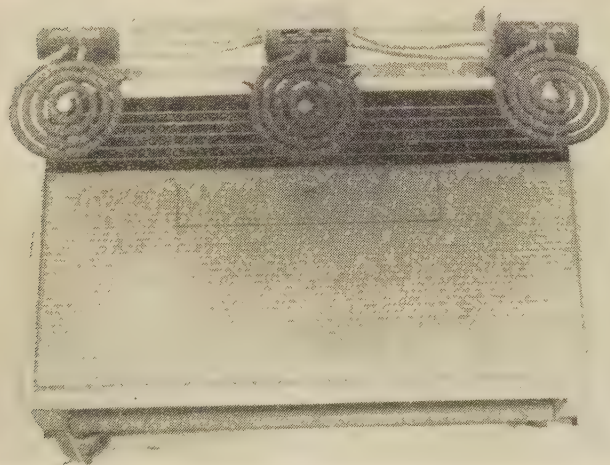


FIGURE 2
Air conditioner and heater units.

Humidity

Humidity control is not always provided in the less elaborate growth rooms but was considered essential for work on cacao. Since the air conditioning normally produces a drop in humidity no special provision is made for dehumidification. To provide high humidities two types of humidifier were tested. The first, a centrifugal humidifier, similar to that used for maintaining high humidities in propagation gave reasonably good humidity control but the air blast was considered too powerful. A

ceiling suspended unit operating on a different principle was found preferable. Control is effected by a paper humidistat operating an electrical contact. Control of humidity to $\pm 2\%$ R.H. is possible during the day but tends to rise at night as outside humidity rises. Fig. 3 shows the two types of humidifier.

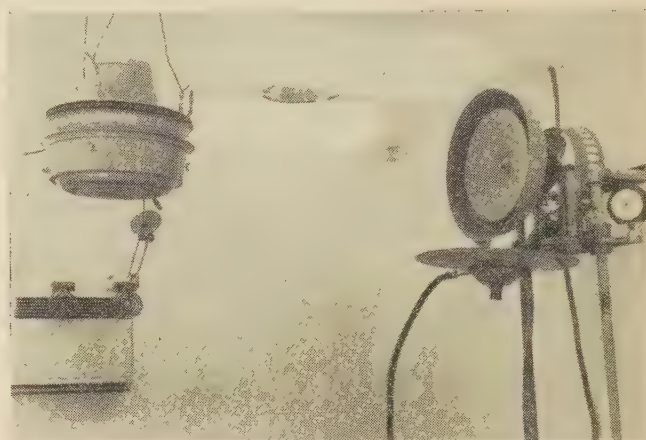


FIGURE 3
Internal view. Bottom left, air conditioner and heaters; top left, ceiling humidifier; centre, fluorescent and fluomeric lamps; right, wall humidifier.

Lighting

The provision of adequate light tends to be a major problem in the growth of plants under artificial conditions. It is not proposed to discuss the problem in detail but to describe the type of lighting that has been designed for this particular purpose. With large numbers of annual crops in the room, uniformity of lighting is required and is usually provided by a bank of fluorescent tubular lamps set in the ceiling. Since the "white" fluorescent lamps are generally deficient in the red end of the spectrum, they are often supplemented by tungsten filament lamps especially where optimum growth is required. In this case use has been made of a dual purpose "Fluomeric" lamp which, combining an incandescent tungsten source with an incandescent tube, gives a spectral composition similar to daylight. The 750-watt lamp is rated at 22,500 lumens and has a life of 6-12,000 hours. Four of these are installed per room vertically above the four experimental plants and are supplemented by 32 4-ft. 40-watt daylight fluorescent tubes. The arrangement may be seen in Fig. 3. To cope with their high heat output the bulbs are set just above the asbestos ceiling and direct their light downwards through a hole; for the same reason the ballasts for the fluorescent tubes are also set in the ceiling area. At 18 in. from the ceiling, a Weston light meter records 2,000 candle power maximum intensity. Since cacao is usually grown under shade at low light intensities, the provision of very high intensities is not considered as important as for other crops. To help with the problem of heat dissipation it is planned to run the lights at night from 9 p.m. to 9 a.m. to take advantage of the lower ambient temperature.

General

Since the whole purpose of the experimental work is to grow plants under as completely uniform conditions as possible, it is obvious that they cannot be grown in soil.

The use of sand was precluded by the large quantity required and the great weight involved in handling the containers. Vermiculite, from experience, loses its structure after six to nine months and this leads to unsatisfactory growth. It was therefore decided to use coconut fibre. Though not inert it breaks down very slowly and it has the advantage of providing good drainage and aeration.

The containers are galvanised iron cylinders of 16 in. diameter and 22 in. depth, with a hole at the bottom for drainage. Two-year-old plants growing in the field were dug up, the roots washed free of soil and planted in the coconut fibre in the containers. They were then placed in a humidifier chamber maintained at 100% relative humidity until well established. This technique proved very successful so that reasonably large plants could be established without set-back. The plants are watered twice daily with Long Ashton nutrient solution. Some trouble has been experienced with iron chlorosis but this has been corrected by attention to pH and use of an iron chelate.

Due to a number of circumstances the setting up of these facilities has taken some considerable time but the experimental programme will shortly be started and the first investigations will cover temperature effects. The effect of both uniform high and low temperatures and of high day-low night temperatures on the cycles of flushing, flowering and cropping will be examined. Such an experiment will probably last a full year. Attention will then be turned to atmospheric humidity effects and later, photoperiod, though this latter is not thought to be of major importance in cacao. Growth at high and low light intensities will be compared and related to field studies of

this factor. Besides full measurement on growth, leaf area, etc., foliar analysis will also be undertaken to secure information in mineral nutrition. It is hoped that these studies will provide in due course a basic understanding of the reaction of the plant in the field to its complex environmental milieu.

Summary

A description is given of the construction and equipping of three controlled environment growth rooms. Facilities are provided for the control of temperature, atmospheric humidity and light intensity and studies will be much of the effect of variations in these factors on cycles of growth, flowering and cropping in cacao.

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*Auxins of cacao and the mechanism which causes the physical symptoms of cherelle wilt

by

R. Nichols

CHERELLE wilt, the shrivelling and blackening of young cacao pods, may account for an appreciable depression in the potential yield of a cacao tree. The figures of Pyke (1933) illustrate the variation between trees with regard to the incidence of cherelle wilt, ranging from a loss of 92% of young pods on some trees to 19% on others. However, this does not mean that in the complete absence of cherelle wilt the crop would be increased by these percentages; clearly it is a physiological impossibility for a normal cacao tree to carry through to maturity all the flowers set and in terms of a practical approach to the problem this point should be fully realised. It follows that cherelle wilt is an intrinsic phenomenon, the end result of which is the maturation of a certain number of pods at the expense of others, the actual relative percentages being peculiar to the physiological status of the tree. It is therefore a reasonable hypothesis to regard cherelle wilt as a physiological thinning mechanism, just as much a part of the overall growth phenomena of cacao as "flushing" or leaf abscission.

The object of this current study has been to determine whether the endogenous growth substances of the developing pods vary with the observed "waves" of wilting and how wilt is initiated. If a correlation between auxins and wilt was shown, experimentation on the use of synthetic growth regulators for the control of wilt would be justified. In this sense control implies a reduction of wilt rather than its complete elimination. In order to clarify this hypothesis it is easier to consider the fate of one individual pod and those factors which may determine the predisposition of this pod to wilt. Assuming normal fertilisation to have taken place, the fate of the pod will depend on a number of factors:—

- (1) Nutrient supply.
- (2) Water supply.
- (3) Endogenous factors peculiar to the pod.
- (4) Freedom from mechanical damage.
- (5) Microbial infection or biotic damage.

It is intended to consider only the first three factors because these may be shown to be associated with wilt phenomena and later to consider factor (4) as a possible contributory factor.

There can be little doubt that nutrient supply must perforce be important to the pod and there is considerable evidence to support this statement. The simplest and most conclusive evidence is that one way of ensuring that the pod will survive beyond the stage at which it will not be subject to physiological wilt, is to remove all other competing pods from the tree and to a limited degree this is used in the practice of carrying out controlled pollination.

What is not known, however, is the maximal number of pods a particular tree will carry without inducing cherelle wilt. The heavy wilt which follows flushing may also suggest that competition for nutrients between the young flushes and the developing pods is at least a partly contributory factor.

The effect of water supply to the pod cannot be separated from the effect of nutrients and it is of interest to note that wilting is equally prevalent in the wet season, when it may be tacitly assumed that the tree as a whole is not under a water stress. This does not preclude the possibility that any one pod may not be under such a stress owing to its intrinsic vascular development and following from this, a consideration of the third factor, endogenous factors peculiar to the pod may be made.

The expression "endogenous factors of the pod" is used to cover certain features of the pod which will endow it with a competitive advantage over its neighbours. Considering two pods only on a tree, if factors (1) and (2) are not limiting then the endogenous auxins may determine which pod will reach maturity by exerting their influence on vascular development, pod wall growth, etc. Clearly with only two pods to consider both may stand an equal chance of reaching maturity but as more and more pods are set so there must come a critical stage in the number of pods on the tree above which wilt may occur through a sub-optimal supply of nutrients or water. It is considered that at this stage the endogenous factors may determine the ultimate fate of the pod by increasing the nutrient flow to the pod thereby conferring upon it a competitive advantage over its neighbours.

The first stage of the problem has been to examine whether there exists in the life history of a pod a predisposition to wilt. It is fairly clearly established that after a pod reaches 10 cm. in length at say 70-100 days from fertilisation it is unlikely to be lost by physiological wilt, that is some physiological stage has been reached which has predetermined the fate of the pod. It may be conjectured that vascular organisation has been completed. Prior to this stage it has been shown by McKelvie (1956) and by Akinbode (1958) that the predisposition of pods to wilt is a discontinuous process in that at 5-6 weeks from pollination a pod is more likely to wilt than at any other time in its development. McKelvie finds two peaks of wilting and Akinbode one but in essence the results are similar. A discontinuous pattern of this type would suggest that auxins may be responsible for the control of the developing pods and the nature of these auxins has been investigated.

A preliminary survey of the auxins of cacao was carried out and a report of this work was published (Nichols, 1957). Three growth substances were found, two acidic and one

* Presented, in part, to the Cacao Conference, Trinidad, 1960; a full account of this work is to be published in *Annals of Botany* (in press).

neutral in behaviour. One of the acid substances behaved similarly in chromatographic investigations to indolyl acetic acid, a commonly occurring natural growth substance, but in the absence of positive identification it was named cacao auxin I. Following this procedure, the neutral growth promoting substance was named cacao auxin II. The other acidic substance was a growth inhibitor, found consistently in all types of cacao tissue examined, *e.g.* cherelles, flush leaves, etc., and a hypothesis was advanced that the phenomenon of cherelle wilt could be explained by assuming that the inhibitor if readily translocated from flushes or pods could inhibit further pod growth. It was shown that the inhibitor could inhibit cell elongation of wheat coleoptiles and that the inhibition was reversible indicating that the inhibitor was not merely acting as a toxic substance. Further development of this hypothesis would demand an identification of the compound or complex; the indications at present are that the inhibitor is a complex of phenolic acids.

A rough quantitative relationship was established between cacao auxin I and pod development, but it awaited the use of the oat curvature test before small quantities of tissue could be used for a quantitative examination of auxins I and II in the cherelles. An inverse relationship between the wilt index and the endogenous auxins of young cherelles was established (Nichols, 1959), *i.e.* free auxins were not readily extractable when the pods were likely to wilt. This was important as it was the first evidence that wilt and the endogenous auxins were correlated and therefore that control by synthetic growth regulators might be practicable.

One of the stumbling blocks in any comprehensive understanding of the problem of wilt has been that the causal mechanism of wilt, *i.e.* the physical expression, was unknown. In many fruits the comparable mechanism is the formation of an abscission layer which effectively seals the fruit from the parent tree. The abscission layer found at the base of the flower stalk of cacao does not become functional once fertilisation has taken place. Unfertilised flowers and delaminated leaves will abscise but not fruits. It is therefore necessary to look for some other mechanism which may effect the physical expression of wilt and this has entailed an anatomical examination of cacao fruits.

The general anatomy of the vegetative parts of cacao has been investigated by Brooks and Guard (1951) but no attention was paid to the fruits. In our investigations the preliminary examination was carried out by mounting pods of various ages by means of their peduncles on manometers filled with basic fuchsin and applying a gas pressure of 5 lbs. per square inch. Within six to eight hours the internal vascular skeleton of the pods is heavily stained and may be examined by macroscopic transverse sections.

Briefly, it can be shown that there are five vascular bundles running centrally through the length of the pod from which lateral bundles are given off to the seeds. External to the seeds are five discrete bundles radial to the central bundles and these also run longitudinally, joining with the central bundles at the basipetal end of the pod. However, between the pod wall and the seeds a distinct pentagonal tissue can be seen macroscopically, a tissue which stains heavily with fuchsin on its inner side. This stained tissue is composed of an indeterminate number of small vascular bundles; the pentagonal layer of tissue which produced these small bundles is an actively dividing cambium. The peculiar feature, however, is that the pod

wall appears to be primarily served by radial bundles contiguous with the small bundles adjacent to the cambial layer but the wall itself does not appear to be served with permanent longitudinal bundles of its own. These observations mean that if the cambial tissue ceases growth the nutrient and water supply to the pod wall will only be maintained for as long as the existing vascular bundles maintain their structure. One of the fundamental differences between a healthy pod and a pod at incipient wilt, is that the cambial layer and tissues internally adjacent to it in a healthy pod exhibit oxidative browning after a few minutes exposure to air, whereas in the pod showing incipient wilt the oxidation has already occurred. It then appears to be a reasonable hypothesis that the cambial layer may be intimately associated with the phenomenon of cherelle wilt either through its capacity to form new vascular tissue or because of its close spatial relationship to the vascular bundles which it produces. However, an examination of the growth curves of a wilting cherelle suggests that the wilt symptoms do not result from a single cause. A cherelle which is about to wilt ceases to elongate and maintains a more or less constant length from 3-6 days (early wilt) followed by rapid shrinkage (late wilt) and it is considered that these two phases, the cessation in growth and the shrinkage, are distinct, but that one "triggers" the other.

Attempts were made to examine the distribution of the vascular tissue of wilting pods in the early and late wilting stages by similar injection methods to those used on the healthy pods. Some infiltration occurred at the early wilting phase but virtually no dye-stuff could be injected into the late wilting pods. The immediate suggestion was that some blockage to liquid flow was apparent in both phases, but that the blockage was more complete at the late wilting stage. Further experimentation showed that if the stalks alone were injected, similar results were obtained, *i.e.* stalks from healthy pods permitted free flow whereas those from wilting pods did not. The cause of the blockage was investigated by preparing stained sections of pod stalks for microscopic examination. Within the vessels of the xylem of the wilting-pod stalks, heavily stained occlusions could readily be seen whereas they were absent from the stalks of healthy pods. Further, in the stalks from early wilting pods, the occlusions could be seen but not with the same frequency as in the late wilting ones. The chemical nature of these occlusions has not been fully investigated although the following points indicate that they are of mucilaginous origin.

(1) In prepared sections, the occlusions present an angular outline, roughly following the shape of the parent vessel. This would indicate that the occluding material originally filled the vessel, but on dehydration by alcohol during the preparation, it shrank away from the wall of the vessel. A hydrated material such as mucilage would be expected to behave in this fashion. In *fresh* sections the occlusions appear as amorphous, translucent bodies within the lumens of the vessels.

(2) The occlusions, after dehydration by alcohol, stain with safranin, similarly in fact to mucilage which has been expressed from a cacao pod and dehydrated with alcohol.

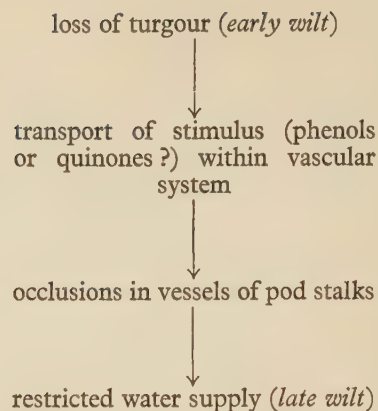
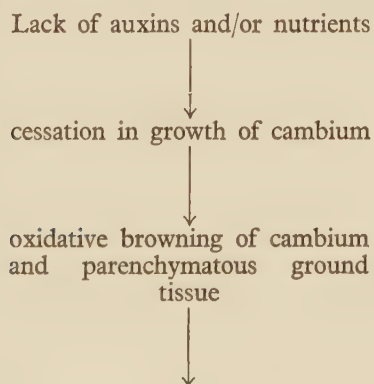
There are other possible explanations, one of which is that the occlusions are tyloses formed by an extrusion of the middle lamella and cell contents from xylem parenchyma cells into the lumens of the vessels. This explanation is unlikely for the following reasons.

(1) Tyloses would be expected to contract irregularly on dehydration, unless the middle lamella had been subsequently thickened. No evidence for such thickening has been observed.

(2) The occlusions have not been shown to be associated with any particular vessel pit, through which the contents of a neighbouring xylem parenchyma cell would have to pass.

(3) Tyloses develop as a result of a difference in hydrostatic pressure between a vessel and a xylem parenchyma cell. Therefore, healthy pods, removed from the tree and allowed to dry naturally, would be expected to form such occlusions if they were of tylosic origin. Healthy pods were allowed to dry on the laboratory bench and periodically examined for occlusions in the stalks; none had formed after 4 days, a period sufficiently long for occlusions to form in pods wilting on the tree.

Irrespective of the chemical nature of the occlusions, it is clear that they are responsible for the sealing of the water conducting elements to the pod but because they develop quantitatively from incipient wilt onwards, they are unlikely to be the *initiating* mechanism of wilt. It is reasonable to infer that the true initiation of wilt starts in the pod and therefore an investigation was made of the anatomy of the tissues associated with the cambial tissue, *i.e.* the tissue which shows precocious oxidative browning in the wilting cherelle. The results of this investigation were that the cambial tissue initiates vascular bundles *de novo* which means that any disturbance of the organisation of the cambial tissue will prevent further vascular bundles being formed. This is important because the vascular bundles of the pod wall itself have been shown to connect with the bundles derived from the cambial tissue. The tissue internal to the cambium but external to the beans is also meristematic because examination of the anatomy of pods of different sizes has shown that the bundles derived from the cambium maintain a relatively constant spatial relationship to one another and to the cambium. From these investigations a mechanism for the physical expression of wilt symptoms may be postulated. Within the young pod, the cambium ceases growth either as a response to lack of auxins from the seeds, or to lack of nutrients. The immediate result, however, will be a cessation in growth followed by a loss of turgour (early wilt) due to the disorganisation of the cambium and subsequent activity of the oxidative enzymes (polyphenol oxidase). Mucilage is subsequently liberated into the vessels of the pod stalks, progressively restricting water supply to the pod until complete drying of the pod ensues. The process may be summarised as follows:—



The mechanism described is compatible with all the factors which have been described as contributory to wilt. For instance, Miller (1954) has shown that mechanical stimulation of young pods will induce them to wilt. External, mechanical abrasion of the pod may be expected to cause an internal disruption of the cambium, triggering the wilt mechanism. Our own experiments have shown that mechanical damage to the tip of the pod results in the initiation of the oxidative activity of the cambium and internal tissues throughout the length of the pod, *i.e.* far in advance of the perception of the stimulus.

It may be argued that the above conception of the wilting mechanism could be applied to older pods which, however, do not exhibit physiological wilt. The explanation of this apparent anomaly probably lies in the difference in the degree of differentiation and distribution of the vascular tissue in old and young pods. It has been shown that lateral rays to the pod wall develop from the cambium and link up with longitudinal bundles on the inside of the cambium. This development takes place as the pod ages and it is tempting to postulate that the change in the "ability" of the pod to wilt coincides with the formation of its final vascular skeleton; that such a change in the vascular skeleton does take place has been shown but the equation with wilting behaviour has to be proved and this point is being investigated.

A number of important conclusions can be drawn from this work.

(1) A simple direct translation of experiments involving the use of growth regulating compounds, which have been used successfully for the control of the fruit drop of other trees, is unlikely to be of any value for the control of cherelle wilt. Fruit drop and cherelle wilt are analogous phenomena, *i.e.* the end results are similar in that the number of fruits which were originally set is reduced but the mechanism by which this is achieved is radically different even though the contributory factors may be the same.

(2) Once wilt of any particular pod commences, attempts to reverse the process are unlikely to be of value; the mechanism is self-accelerating and the chemical nature and location of the occlusions imply that the process will be irreversible.

(3) The type of growth regulating compound which may be effective in controlling wilt is one that will either stimulate vascular flow or cell division.

(4) Application of such a growth regulating compound must be made before visible symptoms of wilt are apparent.

(5) The aims of control measures should be to reduce the degree of wilt by a small percentage, coupled with increased nutrient supply, either as an increased fertiliser application, or preferably as a foliar spray. The object should be to modify the wilting percentage, *i.e.* to permit more pods to pass the wilting stage, such that the available reserves and the additional nutrients can be *utilised*, rather than accept a limitation of the crop, as an expression of the *immediately* available nutrients.

Summary

(i) The auxins of cacao and the interrelation with other factors contributory to cherelle (pod) wilt is discussed.

(ii) A description of the distribution of the vascular bundles in the cacao fruit is given.

(iii) Mucilaginous occlusions occur in the xylem vessels of the stalk of a wilting cherelle and these occlusions have been shown to restrict the flow of liquid to the pod. The symptoms of early and late wilt may be accounted for by these xylem blockages. The initiation of wilt is considered to be associated with the cessation in activity of a meristematic or cambial tissue which forms new vascular bundles to the pericarp of the pod.

(iv) The relationship of these findings with wilt control is discussed.

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Soil moisture and cropping cycles in cacao

by

D. B. Murray

As a measure of the response of cacao to its environment, the total annual crop provides only a very crude yardstick. It has been shown that the annual cropping cycle varies with the age of the plant, the distribution of rainfall and the light intensity (Murray, 1955 a, b). Although the total annual crop is the important economic feature, the rate at which the crop is produced month by month through the season is fundamental to an understanding of the factors affecting total annual production.

In temperate tree crops the fruiting cycle is determined by temperature and/or photoperiod. In tropical tree crops on the other hand rainfall is usually the most important factor. Crops like coffee and citrus tend to have a flowering period related to the dry season and advent of the rains and the resulting crop is reaped at a correspondingly later period of the year. In cacao the problem tends to be more complicated. The tree may flower and fruit all through the year but the production per month is subject to marked maxima and minima. The theoretical question may be posed, other factors being equal, does a tree give the same yield per annum when the crop is secured over a period of say 3-4 months as it would if the crop was produced at a fairly constant rate month by month? How does the distribution of rainfall affect both the total crop and its monthly rate of production?

In order to investigate the effect of soil moisture on the periodicity of cropping a field experiment was planned. There were two aspects of soil moisture to be considered, too little and too much. In the dry season when soil moisture is low the effect of irrigation was sought. Little or no experimental work has been done on irrigation for cacao. On the other hand, in the rainy season, on soils with impeded drainage, excessive rainfall leads to water-logging and poor root development. It has been shown by Dunlop (1925) that a negative correlation exists between rainfall and cacao crop on the heavier clay soils of Trinidad. To overcome this a technique was devised for reducing the amount of rain entering the soil. The cacao was growing on cambered beds with surface drains between; along these beds rolls of waterproof paper were laid so that the greater part of the rain was run off into the drains. Gaps in the paper existed around the trunks of the trees and along the centres of the beds where rain could enter. The arrangement is shown in Fig. 1.

Experimental details

The experimental site was a block of five-year-old cacao which had previously carried an artificial shade experiment. It was now growing without shade. At the conclusion of the shade experiment the trees were given a uniform fertiliser dressing and yield recorded for a year prior to starting the new experiment.



FIGURE 1

Waterproof paper laid on the soil to spill rain into the drains.

Four treatments were imposed:—

- (1) Control.
- (2) Soil covered to reduce rainfall absorption.
- (3) Dry season irrigation.
- (4) A combination of (2) and (3).

The layout was five randomised blocks of the four treatments with 24 trees per plot. The experiment was started at the beginning of the crop year, 1st September, 1955, and irrigation was first applied in the dry season of early 1956. Irrigation facilities were poor, the supply of water coming from low pressure stand-pipes. This was used for furrow irrigation, the drains between the rows of trees being blocked and filled with water once a week.

Soil moisture was measured by gypsum blocks. In each plot two blocks were buried at 6 in. depth and two at 15 in. This gave a total of 80 blocks and resistance readings were taken once a week. The shortcomings of gypsum blocks were realised but it was considered that for long term trends they should give satisfactory comparative measurements. Various difficulties arose however and in 1956 direct measurements of soil moisture by augering to 6 in. were started in addition. By 1957 the soil blocks had begun to deteriorate and they were abandoned.

The first season of furrow irrigation (1956) indicated that this system was not proving very effective and accordingly from the 1957 dry season onwards watering was done from hoses over the cambered beds once a week. In treatment (4) with the soil cover, irrigation was continued by filling the drains only.

The trees were harvested fortnightly and in addition, on three trees per plot, an intensive record was kept of the number of flowers produced, number of cherelles set,

number of cherelles that wilted and mature pods. These records were taken weekly.

Results

The annual production in lbs. dry cacao/acre is given in Table I, and Table II shows the yields in each year as a percentage of the control.

TABLE I
Yields in lbs. dry cacao/acre

Treat-ments	1954-55	1955-56	1956-57	1957-58	1958-59	1959-60
1	1,200	1,540	1,720	1,450	1,760	770
2	940	1,680	1,670	1,410	1,640	690
3	1,120	1,310	1,520	1,420	1,760	1,240
4	1,100	1,380	1,460	1,510	1,770	1,100

TABLE II
Yields as per cent of control

Treat-ments	1954-55	1955-56	1956-57	1957-58	1958-59	1959-60
1	100	100	100	100	100	100
2	79	109	97	97	93	90
3	93	85	88	98	100	161
4	92	89	85	115	101	143

Taking the 1954-55 figure as representing pretreatment yields under uniform treatment the effect of the treatments can best be examined when expressed as a percentage of the controls in each year. In regard to treatment (2), waterproofing the soil, there appears to be a slight increase in relative yield in 1955-56 but over the whole period there is clearly no significant increase in yield as a result of this treatment. Similarly too for treatment (3), dry season irrigation responses were negligible except for the 1959-60 season where a substantial increase over the control was achieved. This occurred however in only one year out of five.

It is necessary to examine the yields in further detail and in Figs. 2, 3, 4 and 5 the crop produced month by month is plotted and also the soil moisture values taken by augering.

The main features that show up from these figures are firstly the changing pattern of cropping year by year irrespective of treatment. In 1956-57 there is a small peak in December and a major one in April. The following season the two peaks are rather similar and in 1958-59 the major peak appears in December. This is typical of the effect of increasing age as previously shown by Murray (1955a).

As regards treatment effects these in general are small and non-significant except for the increased crop in December, 1959, in the two irrigated treatments. This appears to be a result of irrigation in the previous, early 1959, dry season. That season was particularly dry, soil moisture in the non-irrigated plots falling below 10% in the top 6 in. Without irrigation the trees did not appear to recover sufficiently with the rains of 1959 to give the normal December maximum. Irrigation, however, had the effect of maintaining the trees in better condition until the rains started and gave the December peak even though a small one.

That irrigation, however, has by no means compensated for that very severe dry season can be seen from the total yield in that year, Table I. The actual dry season rainfall in 1959 is shown in Table III compared with the other years and the 35-year average.

TABLE III
Dry season rainfall, inches

	1956	1957	1958	1959	1960	35-year Average
Jan.	4.6	2.7	2.3	1.1	1.2	2.9
Feb.	3.0	.8	.9	.5	.5	1.7
Mar.	1.8	.1	.4	.3	.8	1.3
April	2.6	1.9	4.9	.8	3.8	2.0
May	3.1	1.7	13.3	1.9	1.4	4.9
	15.1	7.2	21.8	4.4	7.7	12.8

The results of irrigation are therefore from the practical stand point disappointing. In a normal dry season it has led to no increase in yield over non-irrigated cacao and in a very severe dry season although more crop was produced as compared with the non-irrigated cacao the level of yield was substantially below that of normal years. It has already been stated that the irrigation facilities were poor and the amount of water supplied in treatment (3) could not maintain the soil at the level of field capacity found in the wet season. The soil moisture level in the top 6 in. was however appreciably higher than in the controls. It is thought that another important factor may be low atmospheric humidity in the dry season leading to a rate of transpiration which the roots cannot match even if they are adequately supplied with water. The total leaf area of a cacao tree is high in relation to its root system and there is considerable loss of leaf in an intense dry season. It is difficult to study this effect in the field but experimental work will be undertaken in the controlled environment growth rooms recently installed; the effect on growth and yield of high or low humidity can be examined.

As regards the soil covering treatments, the soil moisture levels were reduced as compared with the controls in the wet seasons. There was, however, an increase in crop in treatment (2) only in December, 1957, though the current wet season was not one of particularly high rainfall. The degree of impeded drainage on the St. Augustine fine sandy loam is not too severe and it would appear that surface drains are adequate to deal with excess rain in the wet season. The possibility of a response on the heavy clays of the Las Hermanas substation is to be investigated.

In view of the lack of a major response to the treatments, examination of the voluminous data on flowering, cherelle wilt, etc., is not justified and the data are not presented. In order to secure further information on the effects of irrigation on cacao a new experiment is being started at the Las Hermanas substation. For this, far better facilities will be available and irrigation will be done with overhead sprinklers using water pumped from the Tumpuna river.

Summary

An experiment is described in which the effect of soil moisture on yield and cropping cycle of cacao was investigated. The treatments included a waterproof cover over the soil to reduce wet season water-logging, dry season irrigation and a combination of the two.

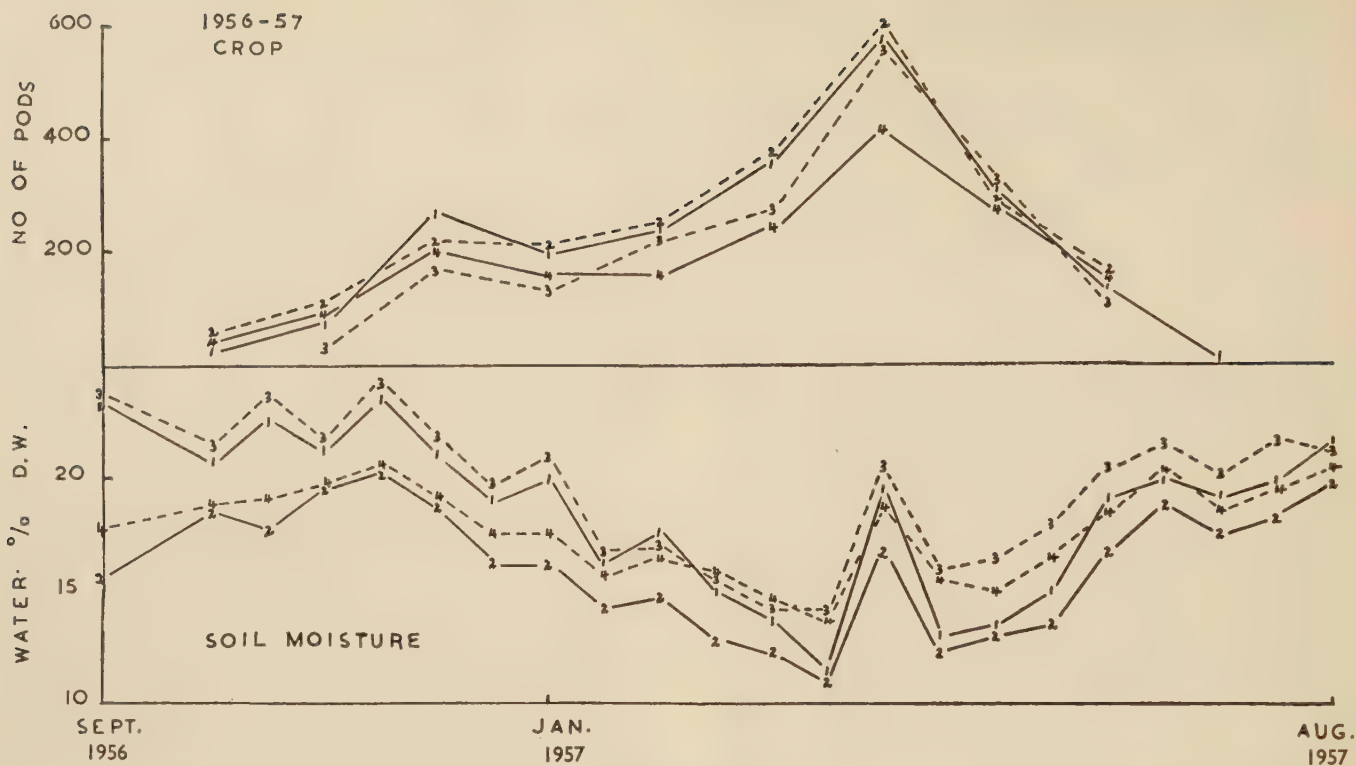


FIGURE 2
Soil moisture and monthly cropping.

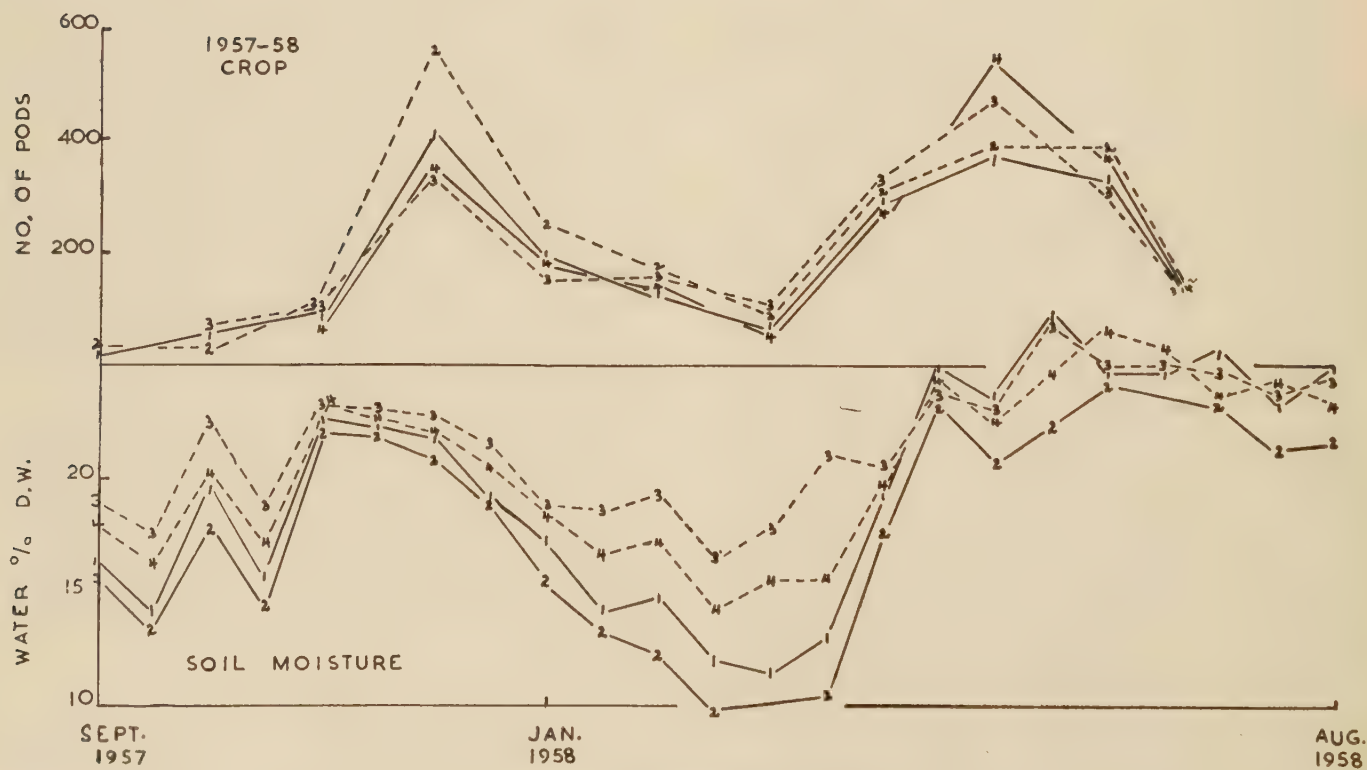


FIGURE 3
Soil moisture and monthly cropping.

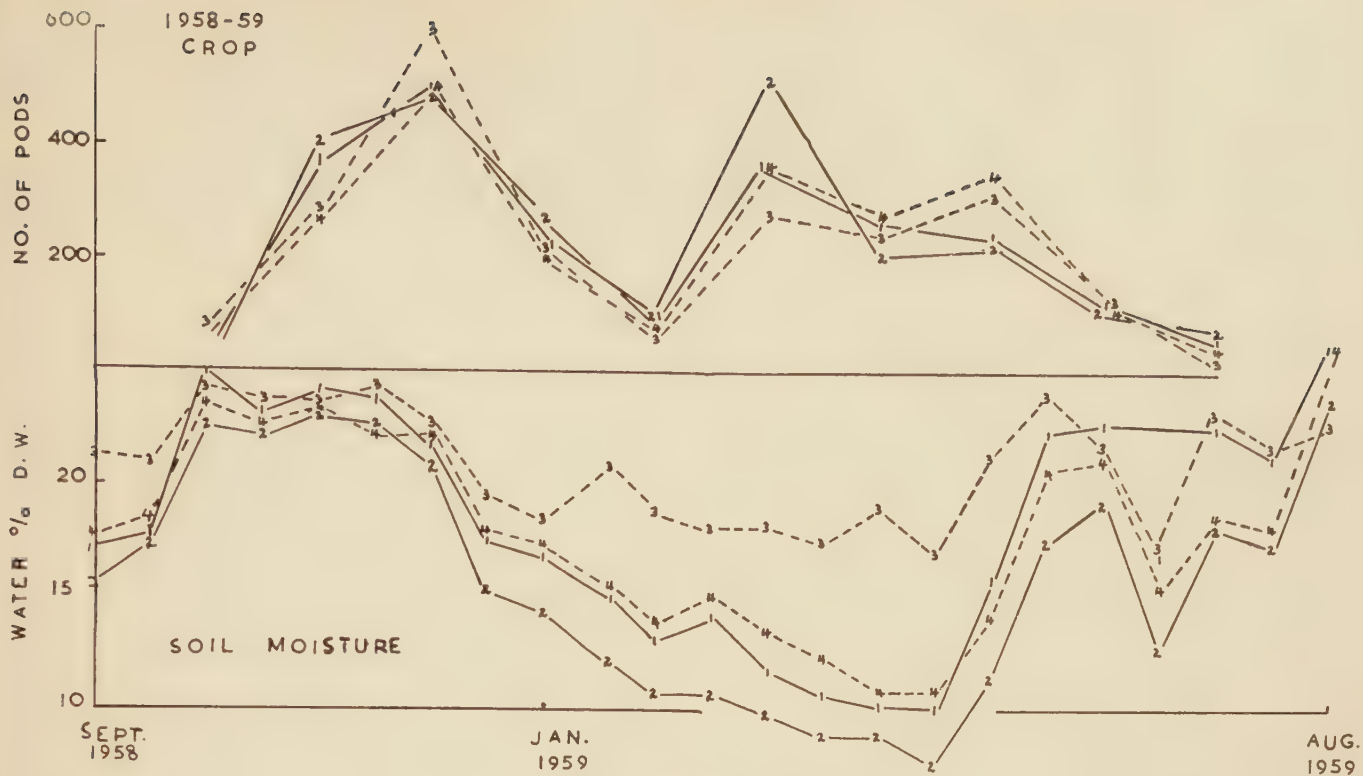


FIGURE 4
Soil moisture and monthly cropping.

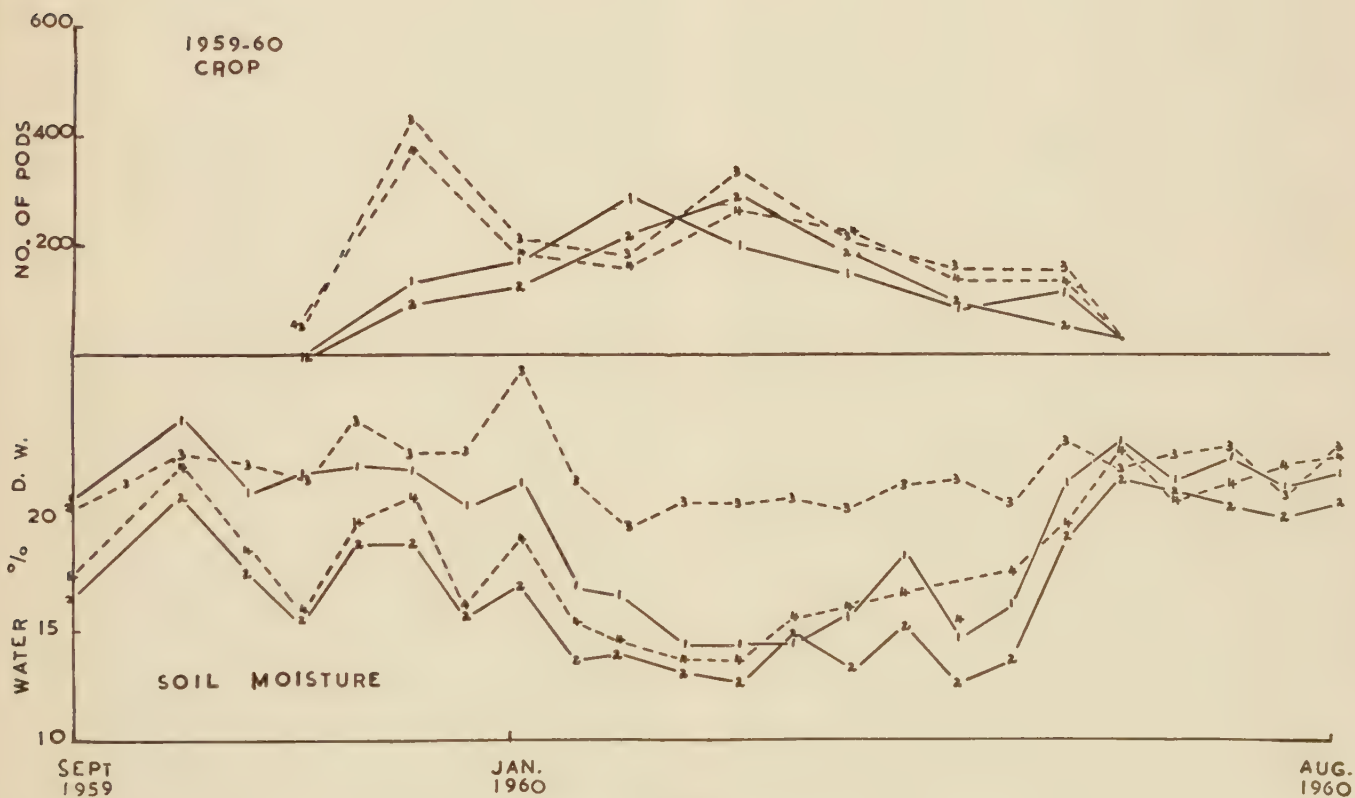


FIGURE 5
Soil moisture and monthly cropping.

No effect was found from the soil cover treatment and with irrigation there was a response in only one year out of five following a very intense dry season. Even in this instance irrigation did not maintain the level of yield previously secured but only modified the depression in yield resulting from the drought. It is suggested that prolonged spells of low atmospheric humidity result in rates of transpiration that the root system, even if reasonably well supplied with water, cannot meet.

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Copper and cacao I. Sand culture studies

by

R. Nichols

Introduction

THE beneficial effect of copper on plant growth under certain conditions has been known for many years (*e.g.* Allison *et al.*, 1927), but the essential role of copper as a plant nutrient was not recognised until the work of Sommer (1931). There has been a considerable amount of work done since that time which has confirmed Sommer's observations but because copper is a constituent of many commercial fungicidal preparations, the effect of the ionic copper on the plant metabolism as a whole may be masked by its fungicidal properties. In a general review of the subject, Coombs (1958) has shown the diversity of crops which respond to trace quantities of copper; some of these are rice, apples, sugarcane, cereals, forage grasses, sugar beet to name but a few. It is important to realise that some of the responses were obtained on heavy soils in which copper was either deficient or in an unavailable form.

The interest in copper and cacao, however, arose from the results of experiments by workers engaged in control of various fungal diseases in which increase in yield was obtained with foliar applications of copper-based fungicides, apparently over and above that expected from the control of the disease alone (Wharton, 1958; De Verteuil, 1958; Tollenaar, 1959). It appeared that the beneficial effect was derived from the copper itself rather than the actual formulation applied because the stimulation in yield was found either with cuprous oxide or Bordeaux mixture although De Verteuil (1958) reported no worthwhile increase in yield with cuprous oxychloride. Some differential effect of copper formulations would be expected owing to absorption differences at the leaf surface. It is noteworthy that the copper effect was recorded irrespective of the type of pathogen for which control measures were being applied, *i.e.* black pod (*Phytophthora palmivora*) in West Africa and Trinidad, or Witches' broom (*Marasmius perniciosus*) in Ecuador. The order of increase in yield (30-43%), including control of black pod, quoted by De Verteuil (*op. cit.*) would present an adequate economic proposition.

An important question arises as to the benefit which may be derived from the application of copper alone, where disease control itself may be uneconomic. On *a priori* grounds, there is reason to believe that copper should be of importance in the metabolism of cocoa. Copper is a constituent of polyphenol oxidase, an important enzyme in the complex biochemical changes which take place during fermentation of the bean and polyphenol oxidase activity has been associated with the physical expression of cherelle wilt (Nichols, 1961 a). Apart from these considerations, further questions arise. If low-volume spraying with copper compounds for disease control becomes an established practice what will be the long-term effects in the

field and at what concentration does copper become toxic and how may the symptoms be recognised?

The object of this study has been principally to investigate the effect of copper on the growth of cacao by the use of sand culture techniques and on yield by low-volume spraying in the field, but at the same time to gather as much information as possible on the effect of copper on the other inorganic constituents of the plant.

Only the more important results will be recorded here because a full account of the work will be published elsewhere. The experiments on low-volume spraying will be published separately.

Sand culture experiments

Forty selfed seedlings of clone ICS 95 were grown in acid-washed sand at four levels of copper in plastic containers (Nichols, 1961 b). The basic nutrient solution given to all plants was the high-nitrogen solution of Hewitt (1952), prepared at half strength with spectrographically pure nutrients for the major elements and full strength for the minor elements. Copper was omitted from the basic solution but was added separately as cupric sulphate according to the experimental requirement. Iron as ferric citrate was added twice weekly; nutrient solutions were changed fortnightly for the first six months and then weekly. The initial levels of copper were 0, 0.005, 0.02, 0.06 parts per million expressed relative to the nutrient solution and there were ten plants of each treatment, one per container.

Zero and trace (0.005 p.p.m.) levels of copper

Deficiency symptoms of copper were first noted 3-4 months from germination on plants receiving zero and on some receiving 0.005 p.p.m. copper. The appearance of symptoms and subsequent morphological effects differed from plant to plant but the general pattern of behaviour described below was the same.

The plants for the first few months were perfectly normal in appearance which may be expected if copper was originally present in the plants; the cotyledons are high in copper (17 p.p.m.; Haworth, 1953) although these were removed once the plants were established. The first copper deficiency symptoms appear on the first leaf of the new flush as a drying and wrinkling of the leaf tip followed by inrolling of the leaf margins; the leaf remains pale green in colour. Once the drying of the tip has started the leaf ceases to lengthen but some lateral growth at the margin ensues, resulting in an ovoid shape rather than the typical lanceolate cacao leaf. The subsequent leaves of the flush exhibit the drying and tip withering symptoms progressively earlier such that these become more and more ovoid, almost as broad as long and boat shaped (Fig. 1a); the youngest



FIGURE 1a

Copper deficient plants, 4 months old. Early stage of symptoms, wrinkling of leaf tip and progressive reduction in leaf size.



FIGURE 1c

Copper deficient plants, 4 months old. Marked loss of apical dominance. The new flush leaves are symptomless but later leaves will develop symptoms and abscise as in (b).

leaves, however, may not expand at all and wither *in situ*. In all affected leaves, tip withering is followed by abscission such that the severely affected plant exhibits above the old flush a stem devoid of leaves although the leaf stipules persist and may remain for a considerable period. The terminal bud, sheathed in leaf stipules and crowning the bare stem, looks like an artist's paint brush. The swelling of the stem (Fig. 1b) at the base of the new flush, first noted by Lockard (1959) was confirmed, although it appeared to be confined to the first copper deficient flush and not to subsequent ones. A few plants (Fig. 1c) showed some loss of apical dominance but this was a variable feature and not all affected plants showed this effect. The plant in Fig. 1d has grown without copper for 13 months. The leaves on alternate "flushes" on the main seedling axis are morphologically normal but were shown to be virtually depleted of polyphenol oxidase. The three regions of leaf abscission at the tip, middle and base of the plant can be clearly seen.



FIGURE 1b

Copper deficient plants, 4 months old. Complete leaf abscission of youngest flush but the leaf stipules persist. Axillary shoot has broken at left of plant. Note swelling of stem opposite ten-inch mark on scale.



FIGURE 1d

Copper deficient plant 13 months old. Note alternate zones of leaf abscission and morphologically normal leaves remaining. The four prominent leaf scars on the top leaf-bearing region of the stem mark the site of leaves removed for analysis.

In order to confirm that the symptoms recorded for copper deficiency were in fact due to low levels of copper and not to extraneous causes, manometric and spectrographic analyses were made on leaves showing deficiency symptoms and on those which were symptomless (*i.e.* the physiologically oldest leaves, but morphologically normal). The technique for the manometric determination was

0.06 to 0.02, 0.06 and 2.0 p.p.m. respectively. There was no change made in the zinc levels but the frequency of complete nutrient change was changed from fortnightly to weekly. Table III records the plant symptoms at 10 months, *i.e.*, 4 months from the copper changes noted above and 4 months from the recording of symptoms shown in Table II.

TABLE I

Copper in bulked leaf and root samples, expressed as p.p.m. dry matter, from plants grown at 3 levels of copper in the nutrient solution. Leaves selected from top and bottom of shoots and analysed separately; results from both plants analysed ((i) and (ii)) are recorded below

	Cu in nutrient solution, p.p.m.								
	0			0.06			2.0		
	(i)	(ii)	Mean	(i)	(ii)	Mean	(i)	(ii)	Mean
Top leaves	2.7	3.4	3.1	4.8	7.9	6.4	11.8	19.3	15.6
Bottom leaves	3.4	4.3	3.9	6.7	8.3	7.5	8.6	7.8	8.2
Mean treatment (all leaves)			3.5			6.9			11.9
Roots	3.4	4.2	3.8	8.4	15.1	11.8	192.6	188.0	190.3

adapted from de Witt (1952) and his observations that the enzymes of copper deficient leaves did not oxidise added polyphenols were confirmed. However, it was also shown that the oldest leaves which were *symptomless* were also unable to oxidise polyphenols or did so slowly and incompletely by comparison with leaves from plants supplied with normal levels of copper, or from field grown plants. The analysis for copper confirmed the low levels present in the leaves of plants supplied with zero copper, see Table I.

It may be seen that leaf symptoms of copper deficiency occur at about 4.0 p.p.m. and that normal levels are from 7.0 to 12.0 p.p.m. These results are not in entire agreement with those of Lockard (1959) who quotes a value of 8.1 p.p.m. Cu for a plant showing copper deficiency symptoms and 9.2 p.p.m. for controls. The high levels of copper in the roots will be discussed later.

Symptoms of zinc deficiencies on plants grown at 0, 0.005, 0.02 and 0.06 p.p.m. Cu.

After six months pronounced zinc deficiency symptoms had occurred in certain treatments. It may be seen from Table II that the severe zinc deficiency symptoms were confined primarily to the 0.005 p.p.m. and 0.02 p.p.m. treatments. The inference from these observations is that when copper is limiting growth, the need for zinc is reduced, but where copper is supplied even at suboptimal quantities the requirement for zinc is increased to the point where a deficiency of zinc may occur. The absence of zinc deficiency symptoms at 0.06 p.p.m. is of interest but may be the result of a fault in technique. Zinc is a common contaminant of copper sulphate and although "Analar" reagent grades of the copper salt were used in this study, there may have been sufficient zinc contamination to prevent the appearance of zinc symptoms. An alternative explanation that copper may in some part offset the need for zinc is a tempting hypothesis but without further evidence will not be pursued. The pH of the nutrient solutions varied between 6.0 and 7.0 and therefore some of the zinc deficiency symptoms may have been partially aggravated by this factor. However, owing to this apparent interrelation of copper and zinc, the levels of copper supplied were changed from 0.005, 0.02 and

The remarkable change in four months was the appearance of marked zinc deficiency symptoms induced by the 2.0 p.p.m. copper treatment and to a lesser extent by 0.06 p.p.m. Cu. Table III cannot be validly compared with Table II owing to the change in the frequency of the renewal of the nutrient solution, *i.e.* although the zinc levels were not altered the total mass presented to the plants was effectively doubled by changing from a fortnightly to a weekly renewal of solution.

TABLE II

Number of plants showing zinc deficiency symptoms:
severe=leaf rolling and narrowing with prominent veins;
mild=leaf narrowing. Eight plants per treatment

Levels of Cu in nutrient solution, p.p.m.	0	0.005	0.02	0.06
Number of plants with severe zinc deficiency symptoms	0	4	4	0
Number of plants with mild zinc deficiency symptoms	1	1	0	1
Normal (for zinc)	7	3	4	7

TABLE III

Number of plants showing zinc deficiency symptoms:
severe=leaf rolling and narrowing with prominent veins;
mild=leaf narrowing. Seven plants per treatment

Levels of Cu in nutrient solution, p.p.m.	0	.02	.06	2.0
Number of plants with severe zinc deficiency symptoms	0	*1	0	6
Number of plants with mild zinc deficiency symptoms	1	0	3	1
Normal (for zinc)	6	6	4	0

* Young leaves growing away from symptoms.

Toxicity levels

Evans and Murray (1953) have described the symptoms of copper toxicity on cacao but give no data on the levels of copper which induced the symptoms they describe. In the experiments recorded previously, 2.0 p.p.m. Cu

was included in the treatments, as the first attempt to induce toxic symptoms. Marked zinc deficiency symptoms were recorded at this level where the zinc concentration had been maintained at 0.06 p.p.m., although normal plants with respect to zinc at the same concentration were produced at sub-optimal levels of copper. It would appear that the *apparent* copper toxicity symptoms were in fact symptoms of zinc deficiency. However, subsequent experiments with levels of copper at 2.0, 5.0, 10 and 50 p.p.m. have so far failed to reveal true toxic symptoms, provided that the zinc supply was adequate, which in these later experiments has been 0.2 p.p.m. zinc.

Effects of copper on dry weight and heights of seedlings

Table IV and Fig. 2 illustrate the pronounced effects of the different copper levels on the dry weights and the heights of the seedlings respectively at different ages. In the absence of copper the plants remain small and stunted. Copper concentrations between 0.02 and 0.06 p.p.m. are sufficient for adequate growth but are probably sub-optimal. Much higher levels of copper can be tolerated provided that zinc is also given in sufficient amount.

TABLE IV

Mean dry weights (gms.) of duplicate plants grown at 4 levels of copper, in relation to age

Copper concentration, p.p.m.	0	0.005	0.02	0.06
Time from germination				
3 weeks	0.4	0.5	0.4	0.5
2 months	1.0	—	—	1.1
4 months	2.1	2.4	2.4	2.7
6 months	5.6	10.5	9.4	8.7
* Copper concentration, p.p.m.	0	0.02	0.06	2.0
11 months	28.8	65.6	80.9	88.0
18 months	68.0	248.0	235.0	129.0

* Copper levels changed in nutrient solution—see text.

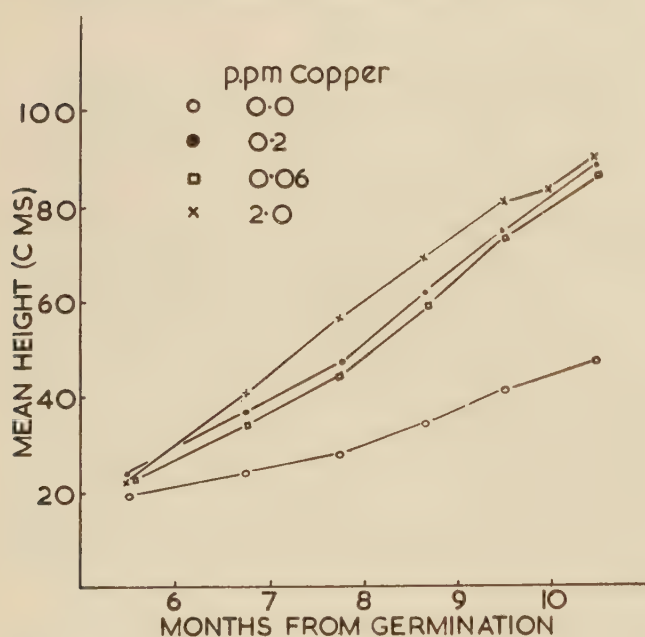


FIGURE 2

Relationship between mean heights of seedlings with age and concentration of copper in the nutrient medium.

Discussion

Important practical considerations arise from these experiments. Symptoms of copper deficiency as described in the text, although clearly defined in sand culture, may never be seen as such in the field. Nevertheless it has been shown that plants may be grown at well below sub-optimal levels and yet show morphologically normal leaves before deficiency symptoms appear; they may then produce normal looking leaves which are still deficient or sub-optimal for copper. The inference from this is that it is quite possible for plants growing under field conditions to require additional copper for normal enzyme production without necessarily showing leaf deficiency symptoms; such a condition would be aggravated by heavy fruit setting.

The experiments with high levels of copper stress the importance of maintaining an ionic balance in sand culture work and the care which must be given to the interpretation of results; it is perhaps fortuitous that zinc deficiency symptoms are easily recognised. The immediate recommendation, however, is that whenever copper is used as a fungicidal spray, zinc should be given as a supplement. These experiments give adequate support for the observations of Tollenaar (1959) that the addition of zinc (as dithane) to mist blown suspensions of copper for control of Witches' broom was beneficial, even though dithane was not itself effective as a fungicide. Clearly, with the increased availability of copper, which presumably must have been limiting yield, an increased demand for zinc was likely. The reason why copper should induce this pronounced zinc effect is not clear and is complicated by the role of copper itself. Copper not only plays an important role in plant metabolism but it also has a marked effect on the oxidation/reduction potentials in the soil (Willis and Piland, 1936) and consequently at the root surface. The tremendous accumulation of copper found in the roots of plants grown at 2.0 p.p.m. Cu suggests that an ionic antagonism with zinc may exist at the root surface, that is, the induced zinc deficiency may be a question of zinc absorption rather than one of zinc metabolism. At all events, an increased supply of zinc in the nutrient medium serves to counteract the effect of the copper.

Acknowledgments

Grateful thanks are due to J. Spector, of the Soils Department of the Regional Research Centre, for the determination of copper by spectrographic analysis and for the supply of spectrographically pure nutrient salts used in the preliminary part of these experiments.

Summary

(i) Cacao seedlings were grown at different levels of copper and the deficiency symptoms are described. Leaf deficiency symptoms of copper are exhibited when the leaf copper content falls to 4.0 p.p.m. expressed on a dry matter basis. Normal levels vary from approximately 7.0 to 12 p.p.m., the lower figure probably being sub-optimal.

(ii) Symptoms of induced *zinc* deficiency symptoms are caused by 2.0 p.p.m. copper where the levels of zinc in the nutrient solution were maintained at 0.06 p.p.m. The importance of copper and the interrelation of copper and zinc in the nutrition of cacao is discussed.

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Containers for growing plants in sand culture

by

R. Nichols

RELATIVELY cheap containers for sand culture work are sometimes difficult to obtain particularly in the tropics and recourse frequently has to be made to locally made clay pots which have to be treated with relatively inert bitumastic paints. Such containers have been used successfully to establish macro and micro nutrient deficiencies although Hewitt (1952) has shown that bitumastic-painted containers may only be used with confidence for 6-12 months owing to breakdown of the paint film. Whether this presents a problem depends on the nature of the investigation but with calcium and micro nutrient studies some resorption from the clay pot may be expected. For long term experiments painted clay pots may not then be satisfactory and in order to investigate the effect of copper deficiency on the growth of cacao (*Theobroma cacao*) in sand culture, "alkathene" buckets have been used, prepared in the following way.

The container is the 1½ gallon plastic bucket obtainable at most hardware stores. The centre of the base usually carries a disc of plastic about 5 mm. thick, left after moulding, through which a hole is punched with a cork borer. The diameter of the hole should be about 7 mm. A length of glass tubing 10 cm. long and 7 mm. in diameter is then heated at one end and a strip of polythene (2 cm. × 15 cm. approximately) is quickly wrapped around the heated end without occluding the bore. The polythene is gently reheated until just molten and the glass tube pushed firmly into the hole at the base of the bucket, polythene-coated end first, such that the glass tube protrudes about 3 mm. through the inside of the bucket. The hot glass melts a small area of the plastic of the bucket which fuses with the polythene and forms a robust, water-tight seal. A thin layer of "Bexol"* is then applied over the glass polythene joint and left to harden. A length of glass tubing a little longer than the height of the bucket is then attached by a short length of P.V.C. tubing to the glass tube sealed into the bucket. A hole is bored into the rim of the bucket with a cork borer, sufficiently wide to accommodate the glass tube. The bucket may then be cleaned with detergent, versenate solution and thoroughly rinsed with suitably purified water. Before filling with sand the open end of the glass tube in the bucket is plugged with acid-washed glass wool. In practice the buckets are supported on a Dexion frame. Buckets prepared in this way have been used constantly for nearly two years with very little further attention.

Flooding with nutrient solution is done by inserting the glass tube into the rim of the bucket and draining by allowing it to hang freely into a suitable container, e.g. a winchester quart bottle. In our experiments, flooding with nutrient solution is done for two half-hourly periods

daily, once in the morning and once in the afternoon. Dust is excluded from the containers by a sheet of polythene stretched over the mouth of the bucket. Holes are pierced in the polythene to permit the plants to grow through. Dust is excluded from the nutrient reservoir by an inverted polythene funnel, with the spout cut to sufficient bore to permit it to run freely up and down the glass tube. Fig. 1 illustrates the containers in use.



FIGURE 1

There is a considerable advantage in using coloured containers because these can be colour-coded according to treatment, thus avoiding nutrients getting into the wrong containers; an important point when long term routine procedures are involved. Although "alkathene" itself is free from inorganic constituents the pigments used in

* Obtainable from "Bexol" Plastics Ltd., Higham Station Avenue, London, E.4.

colouring the buckets may not be and therefore discretion should be exercised concerning the choice of colour. A short and probably incomplete list of pigments containing inorganic ions used in colouring plastic hardware is appended:—

(1) *Lithol reds*

Sodium	...	...	Orange
Barium	...	...	Scarlet
Calcium	...	...	Maroons

(2) *Dry colourants*

Cadmium	...	...	Yellow
Mercury	...	...	Red

Lead chromate	...	Yellow
Chromium oxides	...	Greens

Acknowledgments

The list of inorganic colourants used in plastic hardware was kindly supplied by Modern Methods (Caribbean) Ltd.

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Soil volume and root development in cacao

by

D. B. Murray

THE root system of cacao has not received a great deal of attention, there being little published information other than the work of McCreary *et al.* (1943). In their paper they describe the results of excavations of root systems on various soil types and the distinction is drawn between "physiologically shallow" and "physiologically deep" soils. Shallowness is due to drainage impedence on compact clay soils or to a high water table. Under such conditions the root system is very superficial and confined to the top 6 in. of soil. In "physiologically deep" soils where drainage is free and the water table low the root system is well dispersed. In "physiologically deep" soils a greater soil volume is exploited than in "physiologically shallow" soils.

These excavations were all done on mature seedling cacao. A few excavations done by the author (unpublished data) on trees grown from cuttings have indicated a rather similar development of the root system. A tree grown from a cutting obviously has no tap root but usually two or three leading roots develop from which laterals arise. The presence or absence of a tap root is a morphological distinction which may have little physiological significance.

To investigate further the question of root development in regard to depth of soil it was decided to grow cacao in the same volume of soil arranged to provide a large surface area and a shallow depth, as against a small surface area and greater depth. Wooden boxes were made with internal dimensions of (a) shallow profile— $3\frac{1}{4}$ ft. $\times$ $3\frac{1}{4}$ ft. $\times$ $\frac{3}{4}$ ft. and (b) deep profile— $1\frac{1}{2}$ ft. $\times$ $1\frac{1}{2}$ ft. $\times$ $3\frac{1}{2}$ ft. These boxes would each contain 7.9 cubic feet of soil. Holes in the bottom were provided for drainage and they were filled with River Estate fine sandy loam, a soil which suffers from impeded drainage *in situ*. Before filling the boxes a mixed NPK fertiliser was incorporated with the soil so that nutrition should not be limiting.

There were four deep and four shallow boxes and in each series a cutting of ICS 95 was planted in two boxes and a seedling of the cross SCA 12 $\times$ ICS 1 in two boxes. This cross with an Amazon parent was giving high yields at River Estate and comparison of its early growth against a high yielding cutting was of importance. The boxes were placed in a shaded greenhouse (50% light) and watered lightly twice daily.

Experimental results

The experiment was started on 26th July, 1958, and at three monthly intervals the stem diameter at 3 in. from ground level was measured with callipers and the number of leaves per plant counted. The date of appearance of each new flush of leaves was also recorded.

By 10th October, 1959, when the plants had been growing for 15 months, it was thought that the root system would have penetrated to the furthest point in each box. It was therefore decided to expose and examine the root systems that had developed in each instance.

The procedure adopted was to knock out one side of each box carefully and then to wash away the soil with a hose and thereby expose the root system. The plant was held in place while this was done and an attempt was also made to keep the root system in its relative position by the use of threads strung from side to side of the box. When half the vertical profile had been washed away the root systems were photographed and examples appear in Figs. 1 to 4.



FIGURE 1
Cutting, shallow box.

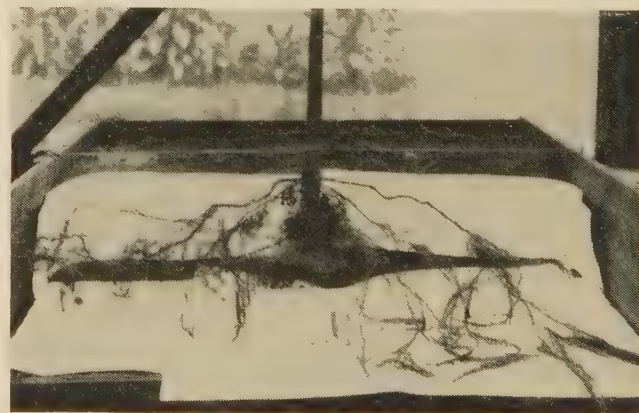


FIGURE 2
Seedling, shallow box.



FIGURE 3
Cutting, deep box.

Fig. 1, the cutting in the shallow box, shows that the root system has spread out to exploit most of the soil volume in the box. The seedling, Fig. 2, has not spread so much and there is a greater density of lateral roots around the tap root. In the deep boxes, the cutting, Fig. 3, has sent roots to the bottom of the box but they tend to follow the sides of the box and the central part of the box contains fewer roots. The tap root of the seedling, Fig. 4, has grown right to the bottom centrally but has produced fewer secondaries than the cuttings.

When the plants had been removed from the boxes a series of measurements were made on them to include:—

- (1) Fresh weight of roots per 3 in. of soil depth from the surface downwards. These roots were further divided into (a) tap root in the case of the seedlings or main roots in the cuttings, and (b) laterals and fibrous roots.
- (2) Fresh weight of stems.
- (3) Fresh weight of leaves.



FIGURE 4
Seedling, deep box.

- (4) Total leaf area, secured by taking leaf punches of known area and calculating leaf area from leaf weight.

TABLE I
Total fresh weight (gms.)

			Cuttings	Seedlings	Total
Shallow	...	...	1,491	1,643	3,134
Deep	...	...	1,213	1,190	2,403
Total	...	...	2,704	2,833	

TABLE II
Roots, fresh weight (gms.)

			Cuttings	Seedlings	Total
Shallow	...	...	468	388	856
Deep	...	...	379	335	714
Total	...	...	847	723	

TABLE III
Stems, fresh weight (gms.)

	Cuttings	Seedlings	Total
Shallow	527	848	1,375
Deep	438	633	1,071
Total	965	1,481	

TABLE IV
Leaves, fresh weight (gms.)

	Cuttings	Seedlings	Total
Shallow	495	462	957
Deep	396	221	617
Total	891	683	

TABLE V
Leaf areas (sq. cms.)

	Cuttings	Seedlings	Total
Shallow	38,900	33,200	72,100
Deep	26,800	17,000	43,800
Total	65,700	50,200	

With so few experimental plants, the results cannot be subjected to statistical analysis but certain general conclusions may be drawn. Both cuttings and seedlings have made better growth, as measured by total weight, in the shallow as compared with the deep profile. There is little difference between the cuttings and seedlings. In regard to the distribution of the weights between roots, stems and leaves the weight of roots developed in the shallow profile is not much greater than in the deep profile. There is, however, an appreciably greater development of stems and leaves on the plants in the shallow boxes as against the deep, the shoot/root ratios being 2.9 and 2.3 respectively. The root system in the shallow profile would therefore appear to be more "efficient" than the root system in the deep profile.

Examining now in more detail the distribution of the root system through the soil volume. In each 3 in. layer from the surface downwards the weight of roots was determined. They were then divided into, in the case of the seedlings, tap root and laterals and in the case of the cuttings, thick main roots and laterals.

From Table VI it may be seen that there is a far higher proportion of weight of tap root to laterals in the seedlings as compared with the proportion of main roots to laterals in the cuttings. In both cuttings and seedlings the total weight of laterals is far higher in the shallow box than in the deep. It will have been noted in Fig. 3, moreover, that more especially with the cuttings the roots tend to

TABLE VI
Fresh weight of tap or main roots (gms.)

Shallow profile			Deep profile		
Depth (in.)	Cuttings	Seedlings	Depth (in.)	Cuttings	Seedlings
0-3	70	125	0-3	73	98
3-6	24	110	3-6	25	67
	94	235	6-9	13	38
			9-12	11	25
			12-18	10	21
			18-24	7	10
			24-30	4	6
			>30	2	3
				145	268

TABLE VII
Fresh weight of laterals (gms.)

Shallow profile			Deep profile		
Depth (in.)	Cuttings	Seedlings	Depth (in.)	Cuttings	Seedlings
0-3	138	77	0-3	39	8
3-6	236	76	3-6	25	7
	374	153	6-9	23	12
			9-12	20	6
			12-18	24	11
			18-24	23	7
			24-30	22	6
			>30	58	10
				234	67

follow the sides of the box where aeration would be expected to be better.

When the weight of laterals in the top 6 in. in both shallow and deep boxes is expressed per square foot of surface area the results in gm. are:

Cuttings			Seedlings
Shallow	...	36	15
Deep	...	29	8

This indicates a greater density of roots in the surface 6 in. in a shallow profile as compared with a deep.

These results, of course, apply only to young plants. It would appear, however, that a shallow profile is more rapidly exploited than a deep. The physiological significance of this needs further attention but would serve to indicate that good aeration is important for early root development hence the importance in the field of an open well drained top soil. Obviously soil conditions in the

boxes are unnatural in that the filling imposes a structure to the soil that would not exist *in situ*. It would be interesting to repeat this work on undisturbed soil profiles but the technical difficulties would be considerable.

Summary

Cuttings and seedlings of cacao were grown in the same volume of soil contained in either a shallow box with a large surface area or a deep box with a small surface area. Better growth was made in the shallow boxes and this was associated with greater top growth rather than root growth. It is suggested that the roots growing in the shallow boxes were more efficient in supporting the top growth. The results emphasise the importance in the field of an open well drained top soil.

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The effect of bark inversions (phloem blocks) on cacao cuttings and seedlings

by

R. Nichols

TREATMENTS which adversely affect the normal metabolism of a tree have long been known to induce early flowering. Some of these treatments are bark ringing, stem strangulation, root pruning, repeated transplanting, summer pruning, pinching and breaking of young shoots (Hensen, 1943; Lindquist, 1948). It was thought that an application of a suitable method to cacao might be useful for the plant breeding programme with the object of shortening the time to the inception of flowering.

Observations that cacao cuttings held in baskets on the nursery floor flowered earlier than similar cuttings in the field suggested that some method which induces dwarfing of the plant might provide a useful practical approach to the problem. Most of the treatments mentioned above do induce some measure of dwarfing but as the final object was to produce a few pods with viable seeds as simply as possible, drastic and laborious treatments such as repeated transplanting were not considered. A modification of the bark ringing method involving bark inversions developed by Sax (1954) for use on apple trees was finally adopted. The problem with bark ringing alone is that normal transport of organic substances is completely interrupted, frequently resulting in the death of the treated limb or the whole tree. In the bark inversion technique, a complete ring of bark is removed from the stem and is replaced upside down into the area from which it was removed. In this way polarity of the phloem of the bark tissue is reversed such that normal basipetal flow of nutrients and auxins is considerably reduced but not to the extent of causing the death of the plant.

Methods

Preliminary experiments were conducted with seedlings and cuttings in baskets in the nursery. A ring of bark half an inch wide was removed from selected seedlings and cuttings by making two parallel cuts around the stems, 4 in. above soil level; the incisions were made into the bark as deep as the central core of wood. A vertical cut was made across the band of bark isolated by the parallel cuts and the whole band was then gently prised off. It was then replaced upside down, *i.e.* with the morphologically lower side of the bark now on the upper or acropetal side; Fig. 1 illustrates this diagrammatically. Since bark consists of the phloem, cortex and epidermal tissues this manipulation has the effect of inverting and therefore reversing, the polarity of the phloem. After the reinsertion of the bark inversion, the treated area was bound with "resinite" budding tape to prevent water loss during the healing together of the tissues; after two to three weeks the tape was removed. The inversion would take quite readily on seedlings and cuttings with stems greater than a quarter of an inch in diameter; on stems



FIGURE 1a

FIGURE 1b

Longitudinal section (not to scale) of stem of a cutting or seedling showing (a) direction of nutrient and auxin flow in phloem before inversion of bark and (b) after inversion of bark.

The arrows indicate the polarity of the phloem and the cross-hatching represents the wood.

smaller than this the superficial damage done to the wood when making the parallel incisions made the plant too fragile for subsequent handling.

After four weeks, extensive callus growth had developed along the suture where the two edges of the inverted bark met. The inverted bark showed little or no further growth but there was considerable swelling above the inversion and, to a much lesser extent, below it.

Cross sections of the inversions showed that new vascular tissue was regenerating beneath the callus formed at the suture and Sax and Dickson (1956) have shown that in apples at least, tissue regenerated in this fashion is polarised in the normal way. Further observations on the treated plants showed that there was a tendency for the bark inversion to be sloughed off by the gradual encroachment and proliferation of the regenerating callus underneath the inversion, although swelling of the upper end of the stem tissue, proximal to the inversion, indicated that an effective phloem block had been caused. However, for the foregoing reasons, this type of blockage may have been expected to be impermanent and therefore a further treatment was included in the experiment, consisting of a second inversion made below the first one. The suture of the second inversion was orientated 180 degrees from the one above in order that even after regeneration with normal polarity, organic substances flowing downwards would still have to pass laterally across the stem between the two inversions. The relative closeness of the two inversions was therefore important and a half inch was found to be the closest that was practical. Plants with one and two inversions are referred to respectively as single and double in the text.

Materials and experimental layout

Originally, cuttings and seedlings from clone ICS 95 were to be used but at the time of the experiments, hybrid

seedlings were becoming available and because of the early promise of this material with respect to yield and Witches' broom resistance, it was decided to use hybrid seedlings instead of seedlings from selfed ICS 95. For the technique to be of any value, it had to be of use on material most likely to be used in the breeding programme. Therefore cuttings of ICS 95 and seedlings of the cross ICS 8×SCA 12 were used. Single and double inversions were made on cuttings and seedlings four to five months of age in the nursery. Plants were selected for uniformity and healthy tissue unions before planting out.

The cuttings and seedlings were planted in October, 1957, at River Estate under established *immortelle* shade in a 6×6 latin square with two plants per plot. The treatments were; single inversions, double inversions and controls of both cuttings and seedlings. Records of girths, heights, time of flowering and cherelle production were kept.

Results

Flowering

Table I shows the number of plants which were in flower in December, 1959, that is when the plants were two and a half years old.

further work was justified. All the remaining plants had flowered by June, 1960.

Heights and girths

The treatments had pronounced effects upon the height of the trees. Seedlings carrying the double inversions were very stunted by comparison with the untreated controls; the treated cuttings showed a reduction in size but not to the same degree as the seedlings (see Table II).

The heights of normal seedlings and girths estimated by the diameter, were significantly greater (1% level) than either those of the single or the double inversions. Measured at 2 years from planting, there was no statistical correlation between the height of the jorquette of the normal seedlings and either diameter or diameter squared, nor was there any correlation between the height of the jorquette and the girth of the seedlings with the single inversion although a positive correlation was found ($r=0.66$; significant at 5%) between the same parameters for the double inversions.

Fruits

By June, 1960, mature pods had been harvested from two normal cuttings and one normal seedling; most of the

TABLE I

Relationship between treatments and number of plants in flower in December, 1959, 26 months from planting; plants approximately 2½ years old

	Cuttings			Seedlings		
	Control	Inversions		Control	Inversions	
		single	double		single	double
Number planted	12	12	12	12	12	12
Number alive	11	6	4	12	12	11
Number flowering	8	2	2	5	1	0
Percentage of plants alive, flowering	73	33	50	42	8	0

Overall, the treatments did not shorten the time of flowering by comparison with the untreated plants. It can be seen from the table that only 10 treated cuttings remained alive out of the original 24; in fact 10 had died by January, 1958. The bark inversion treatment seemed to weaken the cuttings in particular and most of the deaths were from wind damage and subsequent breakage from which the plants never recovered. However, the results from the few cuttings remaining did not suggest that

cherelles set on the treated trees were lost by cherelle wilt. By January, 1961, mature pods were harvested on 6 out of the 10 normal seedlings and on one of the seedlings with double inversions but none was recorded on the plants with the single inversion.

Discussion

The bark inversions do not appear to induce early flowering on cuttings and seedlings but they do induce a

TABLE II

Mean heights and diameters of cuttings and seedlings with and without bark inversions at 2½ years old

	Cuttings			Seedlings		
	Control	Inversions		Control	Inversions	
		single	double		single	double
Mean height cms.	132	118	113	222	140	134
Mean diameter cms.	2.9	2.5	2.6	4.5	2.9	3.1

pronounced stunting effect. Clearly the treatments were successful in one sense in that the phloem blocks had effectively disturbed the metabolism of the tree as a whole but the desirable effect of earlier flowering did not result from it. In fact, the conclusion from this work as far as the seedlings are concerned is that earliness of flowering and fruiting is more likely to be an expression of the vigour of the tree. Mention has been made that hybrid seedlings were deliberately chosen as experimental material; whether seedlings of less vigorous habit would have responded to treatment is a doubtful point but at the best it may be considered only an academic one. The results with cuttings are less well defined owing to the early loss of a large number of the treated trees in the field. Here again, for the technique to be of value, it must be of relatively easy application and if extreme conditions of cultivation are required such as constant irrigation or elaborate windbreaks, any desirable end-result may be offset on economic grounds; as it was, the hybrid seedlings were bearing as early as the cuttings and on these grounds alone the work on cuttings need not be pursued.

No further experimentation is considered worthwhile along these lines. If the treatments had not induced the dwarfing effect, then different treatments such as root pruning, stem strangulation, etc., might have been justified. In fact, by far the most profitable approach to an under-

standing of the flowering and fruiting behaviour of cacao will be through the use of controlled environment rooms now in operation at the college.

Summary

Single and double bark inversions (phloem blocks) were made on cacao seedlings and cuttings and the effect on growth and flowering behaviour compared with untreated plants. The treatments imposed a severe dwarfing effect particularly on the seedlings but no evidence of early flowering was found.

Acknowledgments

Thanks are due to Dr. S. Emsweller for suggesting this work and for advice during it.

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Systems of training cacao grown from cuttings

by

D. B. Murray

THE early growth of seedling cacao presents no problems in training being dictated by the natural habit of growth. No branching occurs before the jorquette develops and at this stage all that is necessary is to remove any branches in excess of the desired number of three or four which then develop to give the scaffold of the tree. The normal fan cutting, however, tends to branch early in the field and this habit of growth requires manipulation to produce a mature tree of a shape and size that allows access between the trees and permits ease of harvesting.

When the use of cuttings as planting material became widespread a system of training had to be developed along *ad hoc* lines and the usual practice recommended was to allow three or four branches to develop low down on the cutting which is normally planted at an angle in the ground. In the course of time these would develop to produce the main system of branches in the form of an inverted cone. The more symmetrical the early development of branches the more likely is it for a well-balanced tree to result.

Another problem exists in regard to chupons arising at the base of the cutting. Little is yet known as to what differentiates fan and chupon habits of growth in cacao but the fact is that buds often develop at the base of a cutting in the field and grow into chupons. The tendency to produce these basal chupons varies with the clone and may be accentuated by mechanical damage, *i.e.* cutlass wounds, cacao-beetle attack, etc. Should a single basal chupon be allowed to develop it tends to supplant the original fan growth and if this is eventually pruned away the result is a tree with the chupon habit of a seedling.

Comparisons have already been made (Cope and Dodds, 1951; Cope, 1953) of the yields from fan cuttings and chupon cuttings, and except for occasional clones in certain seasons no significant differences were found between the yields given by the two differently shaped types of tree. In practice owing to the difficulty of securing chupon material for cuttings as compared with fan material nearly all plantings are made with fan cuttings. Some planters preferring the appearance of a chupon type tree encourage the development of a single basal chupon more especially on steeply sloping land where they claim the chupon root system with its tap root reduces the likelihood of the plant falling over.

Finally, attempts have been made by staking the young cutting to hold it upright and encourage terminal growth only. The intention is to secure a tree with the unbranched appearance of a seedling or chupon. All branches that appear low down are removed and branching only permitted at a height of some 2 or 3 ft.

With this background an experiment was planned to compare—

- A. Controls. Allowed to develop as fan and/or chupon tree.
- B. Fan tree. Chupons removed as they appear.
- C. Chupon tree. One basal chupon allowed to develop and the original fan growth removed.
- D. Cordons. Development of trees allowed in one plane only.
- E. Trunked fan tree. No branching allowed below 2 ft.

The experimental site was Field 6 at the Imperial College of Tropical Agriculture, a field that had been under annual cropping for many years and was under a mixed weed cover. Cocoa is seldom if ever planted on land that has received any cultural treatment, the land being undisturbed except for the planting hole. In this instance it was decided to adopt the unusual practice of preparing the field by mechanical methods.

After ploughing and sub-soiling twice with a Ferguson tractor the land was formed into beds with an Adams grader. Normally on heavy soils in Trinidad, cocoa is planted on beds 24 ft. wide from drain to drain. In this instance the beds were made 9 ft. apart to carry a single line of cocoa down the centre. A 9-ft. spacing rather than 12-ft. was adopted in order to fit the experiment into the field.

A departure from local custom was also made in regard to shade. As temporary shade *Leucaena glauca* and *Cassia reticulata* were planted and this was to give way to *Pentaclethra macroloba*, the Devil's ear, as the final permanent shade.

The layout comprised four randomised blocks of the five treatments. The normal spacing was 9 ft. × 8.2 ft. except for the cordon treatments where the plants were spaced far apart in the rows, 14.4 ft., and the rows closer together at 4.7 ft. Each plot was surrounded by a single guard row. Owing to the different spacing of the cordons the plot size varied from the normal and had to be corrected for in comparing treatment yields.

The experiment was planted in 1952 with $\frac{1}{4}$ lb. mixed NPK fertiliser per hole. The following dry season proved very severe, however, and the loss of plants was nearly 30%. As a result the experiment was replanted the following year and establishment this time was good.

The *Leucaena* made very poor growth and showed no promise under Trinidad conditions. Half the *Cassia* was cut out in 1954 and the balance in 1955. In 1956 the Devil's ear was thinned and again in 1957 to its final stand. This tree appears to give satisfactory shade conditions but grows into a very large tree.

The treatments were maintained as laid down and the cordons were trained by tying the lateral branches to *Gliricidia* stakes, see Fig. 1.



FIGURE 1
Training as cordons.

Experimental results

Some of the trees came into bearing in their third year in the field and 5 years' yields are now available. These are given in Table I.

TABLE I
Yields (lbs. dry cacao/acre)

Treatments	Years old					Total
	3	4	5	6	7	
A	130	1,040	730	1,510	390	3,800
B	90	860	600	1,270	570	3,390
C	100	860	870	1,460	560	3,850
D	70	160	120	220	40	610
E	5	320	340	930	430	2,025

The statistical analysis confirms the superiority of treatments A, B and C over D and E. A, B and C do not differ significantly.

The effect of the treatments is shown more clearly in Fig. 2. Seasonal fluctuation is large, more especially in the 7th year, a bad year with a severe dry season. It is obvious that the severe manipulation and pruning to train the cordons has reduced yields very considerably and this system of training is of no practical importance. The trunked tree, D, also suffers in the early years from the pruning required to maintain its shape but once this is achieved the yields begin to approach those of the control A. Since there is no effect on yield of allowing a single chupon to develop or to maintain the tree as a fan this can be a matter of individual choice. There is, however, one practical feature of a fan cutting in relation to attack by the cocoa beetle, *Steirastoma breve*. When this is prevalent and not well controlled it may practically ring-

bark a chupon type tree and result in death or severe setback. Should it, however, ring one of the low branches of a fan tree the effect is not nearly so serious.

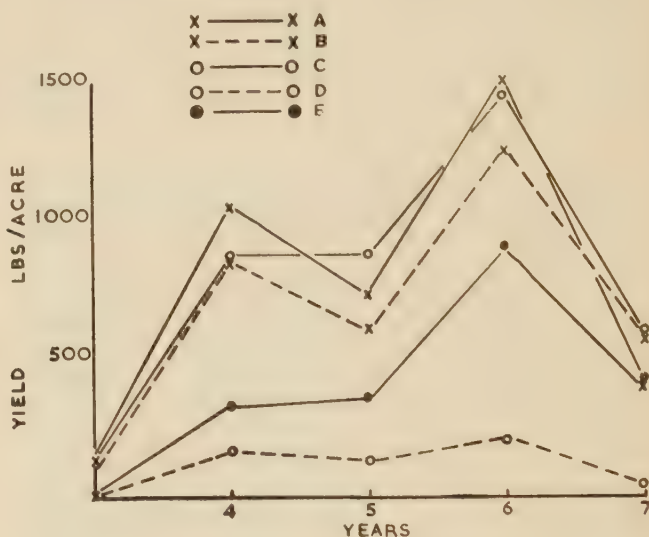


FIGURE 2
Yield in lbs. dry cacao per acre under different systems of training.



FIGURE 3
A well trained tree from a fan cutting.

The general conclusion therefore is that any drastic form of pruning young plants reduces early yields. The shaping of young cuttings should involve the removal of the minimum of young branches to ensure the development of a framework which eventually becomes the main scaffold of the tree. Fig. 3 shows a well trained fan tree. Pruning practice will also be determined to some extent by the shade under which the young trees are growing. Under light shade growth is compact and bushy and less pruning is required than where shade is over-heavy resulting in long lax branches which tend to hang down.

Summary

An experiment is described in which systems of training

young cacao cuttings are compared. Drastic pruning reduces early yields and should be kept to a minimum. The best system is to allow the development of a framework of branches which will give a tree in the shape of an inverted cone.

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A note on chemical weed control in young cacao fields

by

D. Walmsley

LITTLE information is available on chemical weed control in cocoa fields and since this may prove to be the most suitable method of control several weedkillers have been tested under these conditions. A chemical that would be effective against weeds without damaging bananas (used as temporary shade) or cocoa plants was required.

The main weeds present in the trial area were: *Adiantum* spp., *Alternanthera* spp., *Commelina elegans*, *Cyperus* spp.,

bananas (Roshier, private communication). Table I gives a list of the materials tested.

Method

Plots, 24 ft. × 24 ft., containing nine trees, were marked out in a field of two-year-old cocoa. The weeds throughout the field were cut down to ground level and the appropriate weedkillers applied when the weed growth was about 6 in. high and again two months later.

TABLE I

Trade Mark	Active Ingredient	Amount of active ingredient in commercial product	Dose (of commercial product)	Diluent	Volume of Diluent used
Dowpon	Sodium salt of 2, 2-dichloropropionic acid	85%	4, 8, 16, 24, 32 and 48 lbs./acre	Water	75 Imp. gals./acre
Weedicide 100 ...	Sodium arsenite	10 lbs. As ₂ O ₃ /Imp. gal.	$\frac{1}{4}$, $\frac{1}{2}$ and 1 Imp. gals./acre	Water	75 Imp. gals./acre
Shell Q**	pentachlorophenol	15%	2, 4 and 8 Imp. gals./acre 2, 4 and 8 Imp. gals./acre	Water Diesoline	75 Imp. gals./acre 55 Imp. gals./acre
Weedazol*	3-amino-1, 2, 4-triazole (amitrol)	50%	4, 8 and 16 lbs./acre	Water	75 Imp. gals./acre
ACP-M-616A* ...	3-amino-1, 2, 4-triazole (different formulation)	50%	4, 8 and 16 lbs./acre	Water	75 Imp. gals./acre
Fenac*	Sodium salt of 2, 3, 6-trichlorophenylacetic acid	1½ lbs./U.S. gal.	1.1, 2.2 and 4.4 Imp. gals./acre	Water	75 Imp. gals./acre
Fenac* plus Shell Q**			Shell Q at 4 Imp. gals./acre + Fenac at 1.1, 2.2 and 4.4 Imp. gals./acre	Water	75 Imp. gals./acre

* Kindly supplied by Amchem Products Inc.

** Kindly supplied by Shell Trinidad Ltd.

Paspalum fasciculatum (Bamboo grass), and *Scleria melaleuca*.

Materials

No hormone weedkillers were tested since the weed population was mixed and also it is known that weedkillers of the 2:4D type have a detrimental effect on

Observations

An assessment (on a scale 0-10) of the effectiveness of the weedkillers was made at monthly intervals after the second spraying, the unsprayed areas of the field being used for comparison. Any damage to cocoa and banana plants was also noted. These results are recorded in Table II.

TABLE II

Weedkiller	Dose	Effect on Weeds			Damage to Cocoa	Damage to Bananas	Suitable Applications
		1 month	2 months	3 months			
Dowpon	4 lbs./acre	4	3	5	o	o	
	8 "	6	6	6	o	o	
	16 "	8	7	7	+	o	
	24 "	9	9	8	++	+	
	32 "	9	9	8	++	+	
	48 "	9	9	8	---	—	
Shell Q (in water) ...	2 gals./acre	5	4	3	o	o	
	4 "	7	7	7	o	o	*
	8 "	8	7	7	o	o	*
Shell Q (in diesoline) ...	2 "	5	5	4	o	o	
	4 "	6	6	6	o	o	
	8 "	7	6	6	+	o	
Weedicide 100	$\frac{1}{2}$ "	8	7	7	o	o	*
	$\frac{1}{2}$ "	8	9	8	o	o	*
	1 "	8	8	7	o	o	*
Fenac (+4 gal./acre Shell Q)	1.1 gals./acre	8	8	7	++	o	
	2.2 "	9	7	9	++	o	
	4.4 "	9	9	8	++	+	
Weedazol	4 lbs./acre	4	5	o	+	o	
	8 "	6	5	6	+	o	
	16 "	6	7	6	+	o	
ACP-M-616A	4 "	7	7	8	o	o	*
	8 "	7	6	7	+	o	
	16 "	7	7	8	+	o	
Fenac	1.1 gals./acre	6	6	6	o	o	
	2.2 "	7	6	6	o	o	
	4.4 "	7	7	6	++	+	

Weedazol (and ACP-M-616A) did not appear to cause serious damage to the trees. The only symptom was a bleaching of the leaves but new flushes formed subsequent to spraying were quite normal in appearance. Dowpon also produced this bleaching effect but in addition, at high doses, the trees showed abnormal flushing and curled leaves. These latter effects were also apparent on trees in plots sprayed with Fenac at high concentration and when it was used in conjunction with Shell Q. Flushes developed not from the apical buds, which appeared to be destroyed, but from axial buds.

Conclusions

From Table II, considering a figure of seven as showing adequate weed control, only three of the weedkillers tested are suitable, *i.e.* they give effective control of weeds for at least three months without any adverse effect on cocoa or bananas. These are:

Shell Q, Weedicide 100, and ACP-M-616A.

Since Weedicide contains arsenic it must be used with great caution and is not recommended for use with cocoa. Shell Q is now being used successfully for weed control in newly planted cocoa.

The formation of acetic acid in cacao fermentation and its relationship to anthocyanin breakdown

by

L. A. Griffiths

ALTHOUGH acetic acid has long been recognised as one of the major microbial products formed during the fermentation of cacao, quantitative studies on the pattern of synthesis and on the factors influencing synthesis in commercial and experimental microbial fermentation have not previously been undertaken. The significance of acetic acid in cacao fermentation has recently been discussed (Bridgeland and Friend, 1957), but as the experimental basis for their proposals was restricted to measurements of pH as an index of acetic acid formation their value is limited. Recent studies by Quesnel (1957) and Griffiths (1959) on laboratory-scale fermentation techniques have emphasised the importance of acetic acid in the development of the basic flavour characteristics of cacao and have indicated the need for a more complete understanding of the changes occurring in the course of a typical estate fermentation.

Experimental methods

Determination of total volatile acid

50 g. of cacao beans with adhering tissues were macerated in 400 ml. of distilled water. The suspension was made up to 500 ml. with distilled water and an aliquot of 25 ml. taken. After the addition of 1 ml. of syrupy ortho-phosphoric acid, the sample was subjected to steam distillation. Steam, generated in a 3 l. copper vessel containing vigorously boiling water, was passed through the 25 ml. sample in a long necked 100 ml. flask. The distillate was collected by means of a water-cooled condenser in a flask surrounded by a freezing mixture. 150 ml. of distillate were obtained and transferred to a 200 ml. volumetric flask and made up to volume. 20 ml. of this solution were then withdrawn for titration against 0.01 N sodium hydroxide solution.

Paper chromatography of short chain fatty acids

For the separation and identification of short chain fatty acids in fermenting cacao "pulp" a modification of the technique of Reid and Lederer (1952) was employed. Samples for application to the chromatogram were prepared as follows. 20 ml. of the 200 ml. distillate, obtained as described above, were treated with 0.3 g. of barium hydroxide. After shaking, the suspension was warmed and taken down to dryness. 5 ml. of 5% ammonium oxalate solution were then shaken with the residue and the suspension centrifuged to remove barium oxalate. The supernatant, containing the ammonium salts of the volatile fatty acids present, was then applied to the chromatogram the volume being 0.005 ml. After development with n-butanol-aqueous ammonia, the short chain fatty acids were detected by spraying with the bromophenol blue reagent of Kennedy and Barker (1951).

Determination of anthocyanin

5 g. of peeled cotyledons in 50 ml. of n-butanol saturated with 2N HCl was macerated in an M.S.E. microblender for 5 min. After filtration through a Buchner funnel under suction, 1 ml. of the solution containing the anthocyanins and other polyphenols was applied to a column of the polycaprolactam preparation, Versuchs-producte PP 15/SP, B.A.S.F. with Celite 545 (1 : 2 v/v). The column was developed with the butanol/HCl solvent and the anthocyanin fraction, which formed a single narrow red band, eluted and made up to 25 ml. with further butanol/HCl. By paper chromatography it was shown that this fraction contained both cacao anthocyanins, viz. α -L-arabinosidyl-cyanidin and β -D-galactosidyl-cyanidin.

The total anthocyanin present was determined by measuring the absorption at 545μ in a Unicam spectrophotometer. A standard curve was prepared by dissolving standard amounts of pure arabinosidyl-cyanidin in butanol/HCl and also measuring the absorption at 545μ .

Box fermentation

The traditional Trinidad method of fermentation was employed. Quantities of cacao of 2,000-3,000 lb. were placed in estate boxes of the traditional type, insulated at the top by damp plantain leaves and sacking. "Turning" or mixing of the beans was performed at intervals of 48 hours.

Tray fermentation

This was carried out employing equipment similar to that described by Rohan (1958). Beans to a depth of 4 in. were placed in the trays and submitted to turning at 48-hour intervals. In the original procedure described by Rohan, "turning" is omitted but it was introduced in these experiments to permit of a more exact comparison of the changes occurring under the conditions of greater or lesser anaerobiosis encountered in these two techniques.

Results and discussion

Composition of the volatile fatty acid fraction

The composition of the volatile fatty acid fraction obtained by the steam distillation of fermented beans was shown by paper chromatography, employing a modification of the technique of Reid and Lederer (1952), to consist of a single major component, acetic acid. Other fatty acids were occasionally detected but these were present in trace quantity only.

Acetic acid fermentation in estate boxes

The results of a typical box fermentation are shown in Figs. 1 and 2.

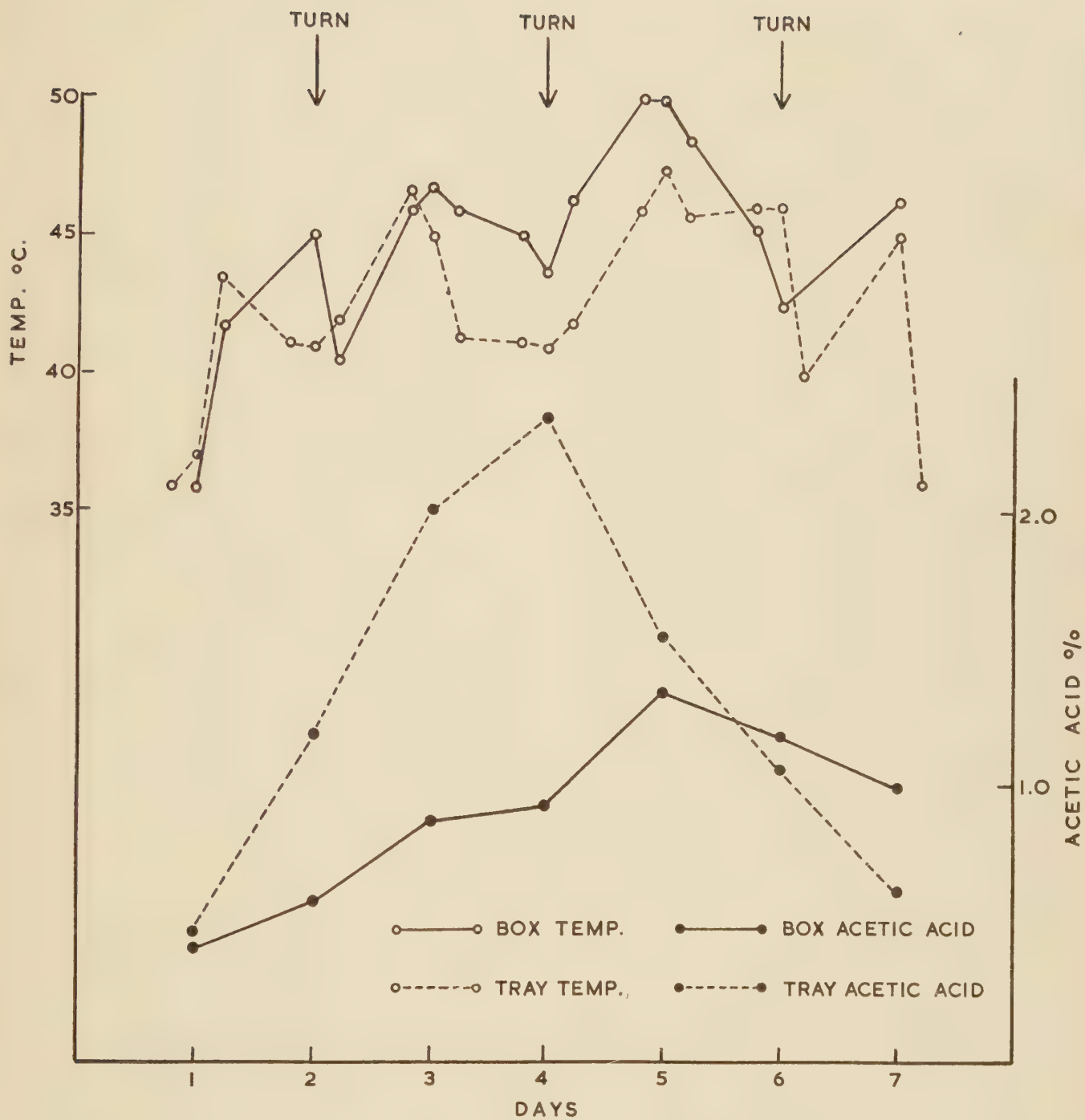


FIGURE I

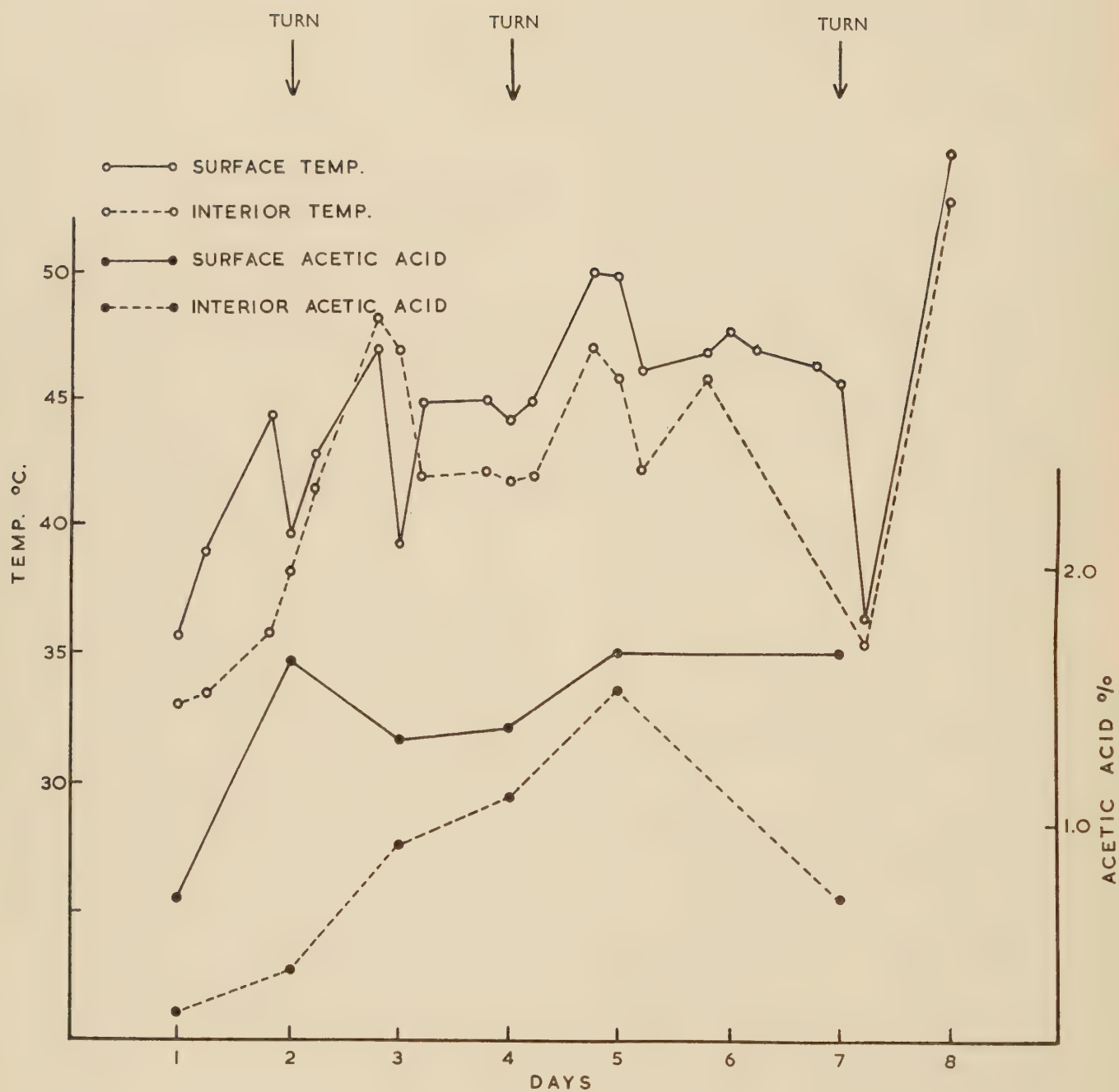


FIGURE 2

Acetic acid formation in boxes reaches a maximum on the fourth or fifth day and afterwards shows a regular decrease. The decrease may be due to depletion of the substrate (*viz.* sugar and ethanol) or death of the *Acetobacter*, as a result of the high temperatures obtaining; cessation of acetic acid formation in the experiment, illustrated in Fig. 1, occurred following the attainment of a temperature of 50.2°C. It is evident that extension of the fermentation period is unlikely to compensate for sub-optimal acetic acid production during the earlier phase.

Since the degree of aeration is known to be of importance in the formation of acetic acid by *Acetobacter*, experiments were undertaken to determine acetic acid levels in the interior of the box where oxygen tension is low compared to those in the surface layer of beans, where a relatively high degree of aeration will obtain. The results obtained are shown in Fig. 2.

It will be noted that although a higher level of acetic acid is maintained in the surface layer throughout the fermentation period, the most marked differences are apparent between the first and second days when acetic acid production in the surface layer rapidly outstrips that occurring in the interior. The low rate of acetic acid formation in the interior during this period is believed to be due to the pulp acting as a barrier to the ingress of oxygen. Following breakdown of the mucilaginous structure of the pulp by the microflora and penetration of the bean mass by the air, oxygen availability would cease to be a limiting factor and acetic acid formation would be accelerated. These changes are, as would be expected, promoted also by "turning." "Turning" on both the second and fourth day is attended by a considerable increase in the rate of acetic acid formation in the interior (Fig. 2). The apparent decrease in acetic acid production in the surface layer following turning on the second day is, however, due to the purely mechanical effect of mixing beans of the surface layer with beans of low acetic acid content from the interior, the reconstituted surface layer after turning containing a high proportion of beans from the centre of the box. The converse effect of an apparent elevation of the interior bean acetic acid level by mixing with the surface layer contributes to a very limited extent to the rise in acetic acid in the interior observed after "turning." This is to be attributed to the high relative volume of the interior or main bean mass to the small volume of the 2-in. surface layer.

Acetic acid formation in trays

It will be seen (Fig. 1) that cacao fermented by the shallow layer tray technique is characterised by a high level of acetic acid formation. In the experiment illustrated in Fig. 1, a level of 2.40% acetic acid was recorded for a tray fermentation on the fourth day compared to only 0.99% in the box type fermentation. Acetic acid levels of 3% and over were frequently recorded for cacao prepared by tray fermentation. The ability of the fermenting mass to attain a high acetic acid level at an early stage in the fermentation undoubtedly accounts for the reduction in the fermentation time reported by Rohan (1958) for the tray technique. It should be noted that cacao removed from trays on the third or fourth day is characterised by very high acidity. It has, however, been found that if subsequent exposure on the drying floor is of sufficient duration much of the excess acetic acid is eliminated before shipping.

Acetic acid formation in a laboratory-scale fermentation

Acetic acid formation in the course of a fermentation, carried out employing the natural inocula technique of Griffiths (1959), was studied. Analyses were carried out at 24-hour intervals following inoculation of the bean mass and the results are presented in Table I. It will be seen that acetic acid levels obtained approach closely to those recorded for the interior of an estate sweat box.

TABLE I

Acidity and anthocyanin content of SCA 6 beans during micro-fermentation employing the natural inoculum method

Sample	Incubation Temperature	Acetic acid % F.W.	mg. anthocyanin per 100 g. F.W.
Fresh bean	—	0.09	90
1st day	30°C.	0.17	80
2nd day	35°C.	0.43	87.5
3rd day	42°C.	0.53	45
4th day	49°C.	0.65	11.25
5th day	49°C.	0.65	—
6th day	49°C.	0.65	—
7th day	49°C.	0.67	—
8th day	49°C.	0.65	—
9th day	49°C.	0.65	—

Localisation of microbially-formed acetic acid in the bean during estate fermentation

The acetic acid levels recorded in Figs. 1 and 2 relate to the acetic acid content of whole beans. It was, however, of interest to determine the relative amounts of acetic acid present in the pulp and the cotyledon at different stages of the fermentation, since it appears probable that the rate of fermentation will depend upon the rate of ingress of acetic acid into the interior of the bean. Analyses by the standard method were therefore carried out on whole beans and washed de-pulped beans from identical samples, the amount of acetic acid in the pulp being obtained by difference. The washing, which was necessary to remove acetic acid from the surface of the cotyledon, was carried out by allowing water to flow rapidly (30-60 seconds) over the beans in a Buchner funnel. Care was taken not to prolong the period of washing to avoid the leaching of acetic acid from the bean interior. The results which are shown in Fig. 3 indicate

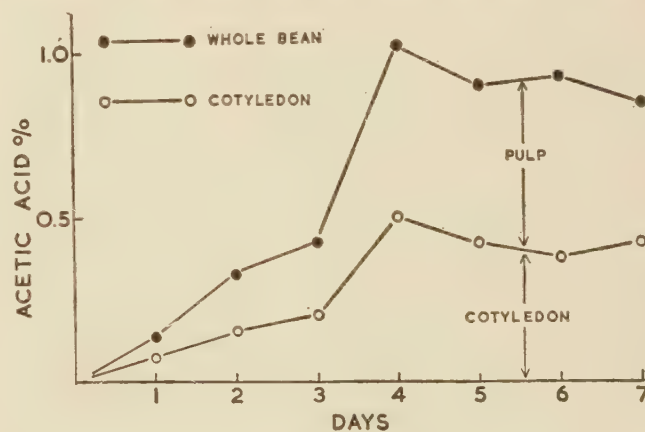


FIGURE 3

that the rise in acetic acid content in the pulp is paralleled by a corresponding rise in the interior of the bean and that

the acetic acid formed is almost equally distributed between the pulp and the cotyledon.

Anthocyanin destruction as a function of acetic acid level and temperature

The breakdown of the anthocyanin pigments in the presence and absence of acetic acid was studied at different temperature levels. In order to achieve an exact control of chemical and physical environmental factors a modification of the technique of Quesnel (1957) was employed, in which the washed, de-pulped beans were incubated in glass jars, containing a range of acetic acid concentrations. The breakdown of the anthocyanins was studied in relation to acid concentration, temperature and time of incubation (Tables II to IV).

TABLE II

Effect of acetic acid concentration on anthocyanin breakdown in SCA 6 beans at a constant temperature of 47°C.

Acetic acid concentration	Content of anthocyanin in mg./100 g. F.W. at		
	0 hrs.	24 hrs.	48 hrs.
%			
0.0	101.25	24.3	12.5
0.2	—	23.8	13.8
0.5	—	13.8	12.5
1.0	—	12.5	3.8
2.0	—	11.3	1.3

TABLE III

Effect of acetic acid concentration on anthocyanin breakdown in SCA 6 beans at a constant temperature of 37°C.

Acetic acid concentration	Content of anthocyanin in mg./100 g. F.W. at			
	0 hrs.	24 hrs.	48 hrs.	72 hrs.
%				
0.0	100.0	80.0	82.5	72.5
0.2	—	85.0	73.0	77.5
0.5	—	67.5	57.5	37.5
1.0	—	62.5	57.5	21.2
2.0	—	30.0	31.2	18.8

At higher temperatures considerable anthocyanin breakdown occurs in the absence of acetic acid but at lower temperatures breakdown of anthocyanin is initiated only in the presence of higher levels of acetic acid. The

maximal rate of anthocyanin breakdown is obtained only when high incubation temperatures and high acetic acid levels are employed.

Considering these results in relation to the sequence of events in the estate sweat box it is evident that early anthocyanin breakdown in the box is induced by acetic acid; the temperature being initially too low to have any marked effect at this stage. Later the breakdown will be largely due to the combined effect of higher temperatures and acetic acid production. It will be seen (Table III) that a temperature of 37°C. held for 72 hours results in only 25% breakdown of the anthocyanin in the absence of acetic acid. In the presence of acetic acid, however, breakdown at this temperature of up to 80% of the total anthocyanin occurs. At 47°C. (Table II) a breakdown of 75% anthocyanin occurs within 24 hours in the absence of acetic acid, whilst appreciably higher values are obtained in the presence of the acid.

Summary

The rate of production of acetic acid in cacao fermenting in a standard sweat box, a Rohan tray and a laboratory micro-fermentary has been investigated and comparisons made. The effect of acetic acid concentration and temperature in the rate of breakdown of anthocyanin pigments in the bean has also been studied. The maximal rate of anthocyanin breakdown, associated with reduction in astringency, occurs when high temperatures and high levels of acetic acid occur together. These are therefore the conditions to be aimed for in normal estate fermentation of the crop.

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TABLE IV

Effect of acetic acid concentration on anthocyanin breakdown in SCA 6 beans at a constant temperature of 30°C.

Acetic acid concentration	Content of anthocyanin in mg./100 g. F.W. at						
	0 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.
%							
0.0	112.5	95.0	110.0	90.0	78.8	90.0	75.0
0.5	—	85.0	82.5	77.5	75.0	73.8	60.0
1.0	—	81.3	77.5	62.5	52.5	45.0	35.0
2.0	—	80.0	82.5	32.5	26.8	24.3	18.8

Studies on a wilt disease of cacao at River Estate

II. Some aspects of wind transmission

by

E. F. Iton

Introduction

In a previous publication (Iton, 1959) the outbreak, distribution and symptoms of a wilt disease of cacao, new to Trinidad and caused by *Ceratostomella fimbriata* (Ell. & Hals.) Elliot, were described. The epidemiology of this disease at River Estate was discussed and evidence was adduced to indicate that it was transmitted, at least in part, by wind.

The problem of disease transmission is important as its thorough comprehension is a pre-requisite for the application of rational measures of control. The practical significance of this is evident as this disease may well become one of the major cacao diseases in the New World, and in this connection its progress in the past decade or so is worth noting.

At River Estate, Trinidad, where this disease broke out in 1958 and where the mortality was 0.7% in January, 1959, the mortality had risen to 5% by the end of 1960 in a population of 90,000 trees. In Colombia the disease was first recorded in 1939 (Garces, 1944) and in 1956 it was described as a serious epiphytotic (Idrobo and Cardenosa, 1956). From that same country Idrobo (1958) reported deaths of up to 50% of the cacao trees in some areas. In Venezuela Malaguti (1958) noted that the disease was destroying the plantations in certain districts where cacao of the Criollo type predominated. Similarly in Mexico, where the disease is serious (Oechsli, 1957), Criollo cacao is the most susceptible. No estimate is available of the seriousness of the disease in Costa Rica, but the disease is endemic there (Siller, 1958). In Ecuador, despite the fact that Desrosiers (1958) considered that the incidence of the disease was of insufficient proportions to cause alarm, combined information from his paper and from Wallenius (1958) indicated a loss of approximately 40,000 trees on one estate alone in less than seven years. Even where the incidence of this disease is low and its spread slow the possibility must not be overlooked that it may become a major disease. van der Plank (1959, 1960) points out that the general rate of spread of systemic diseases of trees is slow but that this does not prevent them from becoming major epidemics. Dutch elm disease which has certain features parallel with *Ceratostomella* wilt has become very important in the United States (Harrar, 1959) and is spreading steadily in other countries such as eastern Canada (Connors, 1956).

This paper deals with an extension of investigations initiated into wind transmission of *Ceratostomella* wilt of cacao (Iton, 1959) and makes special reference to the source of inoculum, its liberation and the inoculum potential. Although a large body of evidence for wind transmission

will be presented there is also evidence for insect transmission (Naundorf *et al.*, 1956; Iton, 1960). Possibly other modes of spread exist. The final assessment of the relative importance of the different methods must therefore await further investigations.

Methods and results

Isolates of *C. fimbriata* cultured on agar media were studied to observe the morphology of its reproductive bodies. Studies of the disease in the field and greenhouse and microscopic examination of the diseased host plants were then linked with the mycological observations in an attempt to elucidate the significance of the spore forms of this pathogen in disease transmission.

Culturing of pathogen

To prepare cultures of the fungus chips of wood were taken from the base of the trunk of a cacao tree recently killed by *Ceratostomella* wilt. These chips were incubated in a moist chamber at laboratory temperature (28-30°C.). Overnight incubation produced a thin grey mat on the wood surface. This mat consisted of mycelium from which arose numerous phialides extruding dry, hyaline, thin-walled, cylindrical endoconidia as shown in Fig. 1. Isolates were made by picking off endoconidia with a sterile needle and inoculating agar slopes. The fungus grew on several media but the most suitable medium used in these investigations was oatmeal agar. Specimens from the cultures were mounted in cotton blue in lactophenol for microscopic examination.

Spore forms

The fungus produces in culture three different asexual spore forms and one sexual spore. The asexual spores are (1) the thin-walled endoconidium, (2) the thick-walled endoconidium, and (3) the very thick-walled chlamydo-spore. The sexual spore is the ascospore.

The thin-walled endoconidia are the first to be produced and also the most abundant. They are borne in chains as in Fig. 1. They measure on the average $12 \times 5\mu$, with a range of $9-16\mu$ in length and $3-6\mu$ in width.

The thick-walled endoconidia are produced next in order. They also are dry spores borne in chains. They range from cylindrical to ovoid and their walls are appreciably thicker than those of the thin-walled endoconidia. Another point of difference from the thin-walled endoconidia is the brown pigmentation of the walls. These endoconidia measure on the average $13 \times 6\mu$ with a range of $9-16\mu$ in length and $4-7\mu$ in width and are shown in Fig. 2.



FIGURE 1

Photomicrograph of *C. fimbriata* showing phialides with thin-walled endoconidia.

The chlamydospores are produced in relatively small numbers in pure culture. They are usually borne singly but occasionally in twos at the end of a hyaline conidiophore. They vary in shape from spherical to ovoid. Their walls are at first thin and hyaline but later become very thick and brown. Their average dimensions are $12 \times 10 \mu$, their length ranging from $9-14 \mu$ and their width from $7-12 \mu$ (Fig. 3).

Perithecia are produced in large numbers in oatmeal agar cultures after about one week (Fig. 4). They arise singly on the surface of the substrate and appear black at low magnifications. High power examination shows, however, that they are dark brown. The body of the perithecium has a diameter of 220μ and height of 170μ . The neck is 625μ long and 25μ in diameter in its middle region. It terminates in a corona of pointed hyaline hyphae surrounding the ostiole.

Each perithecium contains a very large number of ascospores. These have, however, never been observed within asci. They are released in a thick mucilaginous

matrix which oozes slowly out through the ostiole and collects as an irregular mass on the perithecium neck.

The ascospores are hyaline and ellipsoid but each bears a rim which gives it the appearance of a small "bowler hat" (Fig. 5). They measure $6 \times 4 \mu$ on the average, with a range of $5-6 \mu$ in length and $3-4 \mu$ in width.

Inoculation experiments with pure cultures

Inoculations were done on healthy cacao cuttings with a view to determining what spore forms are produced by the pathogen in the host. It had been shown by Naundorf *et al* (1956) and by Malaguti (1958) that the pathogen was a wound parasite. Several inoculation techniques involving wounding were tried but the most successful was the double half-girdle method described below. This produced a much higher percentage of deaths than that previously reported by the author (1959).

Week-old oatmeal agar cultures were used to inoculate cuttings of different clones ranging from 12 to 21 months

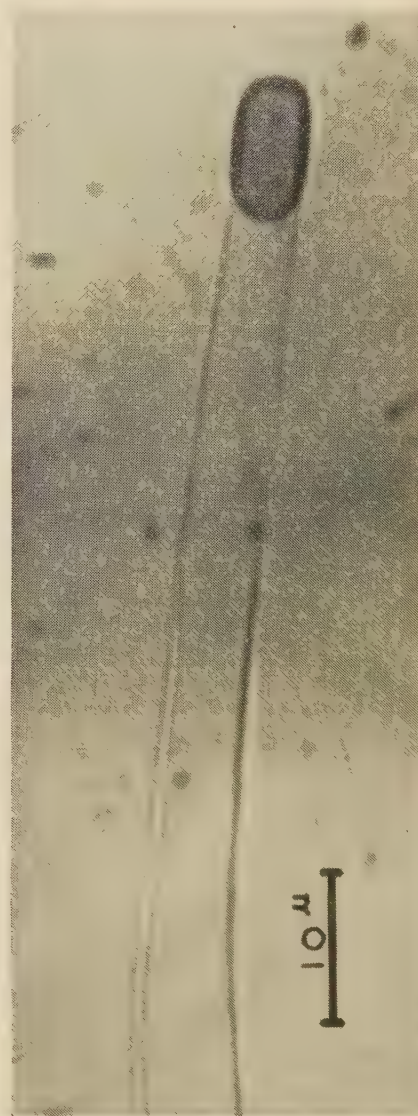


FIGURE 2

Thick-walled endoconidium in phialide.

in age. The inoculum was prepared by removing the surface layer of oatmeal cultures to a depth of about 5 mm. and mixing this thoroughly to obtain a uniform distribution of the fungus in the agar.

The test plants were prepared by cutting two half girdles into the bark on opposite sides of the stem and at heights of approximately 7 cm. and 10 cm., respectively, above soil level. The flaps of bark (approximately 7 mm. $\times$ 5 mm.) were removed, the wood surface thereby exposed was scraped to damage it and a small quantity of the inoculum was applied to it. The tip of a Borradaile needle was found to be a convenient instrument to pick up approximately uniform quantities of inoculum and just enough to cover the exposed surface of the wood. The flaps of bark were replaced and the entire inoculation region was wrapped in a polythene sleeve held in place by rubber bands. In each case an equal number of controls was set up. These were treated exactly as the test plants except that the oatmeal agar applied to the half girdles was sterile. All the plants were kept in a greenhouse and were watered twice daily. Table I shows the results of these experiments.

Examination of inoculated plants

External examination of the test plants, both before and after death, showed that no sporulation of the fungus occurred on the uncovered surface of the stem, although internally the mycelium had extended beyond the area of the stem covered by the polythene sleeve. Abundant sporulation occurred, however, on the surface covered by the polythene sleeve. This region was very moist. On it were numerous perithecia, more or less dense brown mycelial mats bearing numerous chlamydospores on conidiophores, and some grey patches of mycelium bearing thin-walled endoconidia.

It is noteworthy that other fungi were present and that

they also sporulated only on the polythene-covered region of the stem. This indicates that the polythene sleeve functioned as a moist chamber favouring sporulation generally. It suggests that a duplication of the microclimate created under the polythene would induce heavy sporulation on a diseased host surface. The physical conditions in the greenhouse did not permit the formation of reproductive bodies on the exposed surface of the test plants, but it is conceivable that the microclimate at the surface of cacao trees under field conditions could stimulate sporulation on diseased host plants.

Internally the tissues of the inoculated plants were discoloured. Bluish grey and brown streaks showed conspicuously in the wood which is normally white. As was to be expected, the discoloration was at first restricted to the inoculation region but with time it spread. The rate of advance of discoloration appeared to be higher distal to the points of inoculation. There was never, however, complete colonisation of the entire length of the host plant by the pathogen. Hand-cut sections from fresh material were mounted in cotton blue in lactophenol and examined microscopically. Numerous chlamydospores were present intracellularly (Fig. 6) particularly in the vascular rays.



FIGURE 3
Chlamydospore of *C. fimbriata* in culture.



FIGURE 4
Perithecium of *C. fimbriata*.

TABLE I
Pure culture inoculation

Clone	No. of plants	Inoculum	Time for first symptoms (in Days)	Time for death (in Days)	Deaths	
					No.	Percentage
ICS 39	5	<i>C. fimbriata</i>	4—5	7	5	100
	5	<i>Fusarium decemcellulare</i>	—	—	0	0
	5	<i>Fusarium</i> + <i>Ceratostomella</i>	5—6	7	5	100
	5	Sterile oatmeal agar	—	—	0	0
	5	<i>C. fimbriata</i>	12—16	17—20	5	100
	5	<i>F. decemcellulare</i>	—	—	0	0
	5	Sterile oatmeal agar	—	—	0	0
	10	<i>C. fimbriata</i>	6	9—10	10	100
	5	Sterile oatmeal agar	—	—	0	0
	5					



FIGURE 5
Ascospores of *C. fimbriata*.

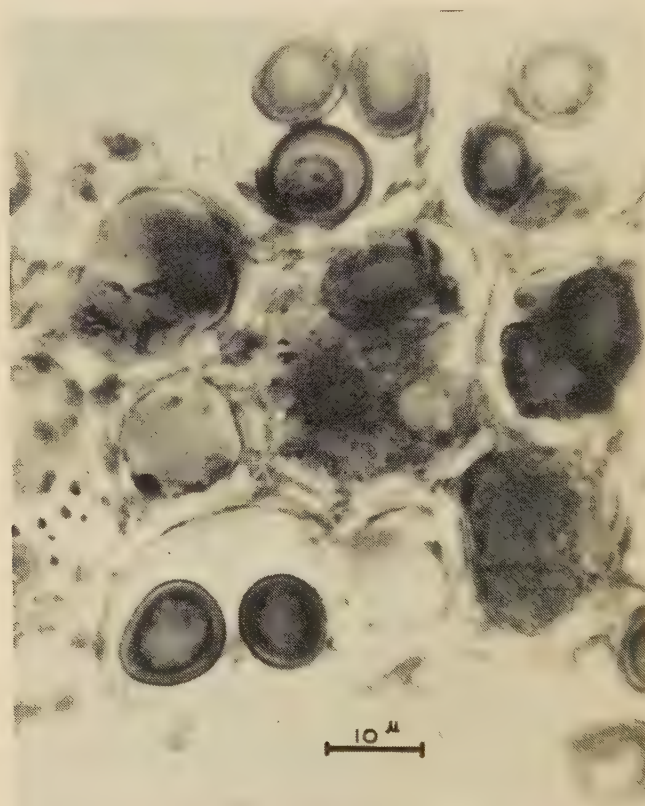


FIGURE 6
Chlamydospores of *C. fimbriata* in cacao wood.

Examination of naturally infected trees

Similar investigations were attempted on naturally infected trees at different stages of wilt in the field. Certain difficulties were encountered, however. The surface of the trunk of cacao trees in the field was quite different from that of the test plants which were cuttings ranging from 12 to 21 months old. Whereas the bark of the test plants was relatively smooth and free of saprophyte colonisation, the bark of mature trees in the field was rough, broken and necrotic, both naturally and from wounds inflicted during cultivation of the crop, and there was a surface flora of several saprophytic fungi which tended to complicate an investigation of this nature.

Notwithstanding this, many examinations of field trees led to the conclusion that the general picture was the same as in the test plants, i.e. sporulation of the wilt pathogen does not occur extensively on the natural surface of the

host plant. While it was not uncommon to isolate the related but innocuous fungus *Ceratocystis paradoxa* (Dade) Moreau from lesions on the surface of pods of otherwise healthy trees, usually no sporulation of *C. fimbriata* was to be found on the pods of wilting or dead trees. Only in one case was the wilt-inducing fungus isolated from lesions found on the surface of a pod. This pod was on a tree which showed no infection in its vegetative parts and the lesion bore only thin-walled endoconidia.

As was to be expected from the formation of thin-walled endoconidia on exposed wood surfaces in the laboratory, as described in the isolation technique above, exposure of infected wood in the field resulted in heavy production of this spore form. Sometimes this spore form was also found on mycelium growing on frass heaps which collected at the gallery entrances of the *Xyleborus* beetles which in-

variably infest *Ceratostomella*-diseased cacao trees at River Estate. During the isolation of the pathogen from diseased wood it was also observed that when wood chips were incubated for 7 to 10 days, appreciable numbers of perithecia and of stalked chlamydospores developed on the wood surface in addition to the thin-walled endoconidia. It is therefore conceivable that perithecia and chlamydospores could be produced on exposed wood surfaces under suitable conditions in nature. They, however, appear to be rare.

Internally, however, there were chlamydospores in abundance situated intracellularly and reaching their highest concentrations in the vascular rays, as was the case in the test plants. High concentrations of chlamydospores were also present in the cells immediately surrounding the *Xyleborus* galleries, and thin-walled endoconidia were abundant on the mycelium lining the beetle galleries.

The wood of the majority of diseased plants in the field is most intensely discoloured in the trunk region at or just above soil level and the discoloration extends for variable distances down into the roots and for about 2 to 3 ft. up into the stem. From its distribution and progress in the inoculated plant it is clear that discoloration is caused by infection, and it is evident that in the field infection usually occurs in the region of the plant axis at about soil level. Chlamydospores are often to be found on hyphae in the lumina of the xylem vessels. It is conceivable that upward translocation of these spores may occur in the transpiration stream thereby enabling rapid colonisation of the wood. The consistent observation that discoloration spreads faster in an upward direction could probably be accounted for by some such mechanism.

Comparison of sporulation patterns in culture and in the host

The difference in the relative proportions of the spore forms in pure culture on the one hand and in the diseased host on the other suggests some difference in significance of the several spore forms in the bionomics of the pathogen in nature. It was remarkable that, although the environment of the artificially inoculated plants in the greenhouse was different from that of the naturally infected plants in the field, in both sets of conditions the thick-walled chlamydospores were relatively abundant in the host whereas this spore form was rare in pure culture. Similarly the high frequency of thin-walled endoconidia on cut wood, on frass and in beetle galleries may be directly related to their importance in the propagation of the fungus in nature.

Examination of frass extruded by Xyleborus borers

As preliminary observations had led to the hypothesis that the disease was wind-transmitted, as spore trapping had revealed the presence of wood dust particles in the air (Iton, 1959) and as sporulation followed the pattern described above, the present investigations centred round the wood dust extruded by the *Xyleborus* beetles, the species of which are listed elsewhere (Iton and Conway, 1961). In this paper the term frass is used for this wood dust and does not refer to insect excrement.

In view of the great abundance of chlamydospores in the cells surrounding the borer galleries it seemed reasonable to expect a similar high density of chlamydospores in the frass. Microscopic examination of the frass confirmed this. The chlamydospores were present both intracellularly in the finely divided bits of wood and also lying free between the particles. Sometimes fair numbers of thin-walled endoconidia were also present as free units in fresh frass.

In previous investigations (Iton, 1959) chlamydospores were found in microscopic bits of wood on greased slides suspended vertically near diseased trees in the field. Failure to find free spores on these traps may be due to the inefficiency of such simple traps to collect individual spores of that small size (Gregory, 1950; 1952). It was therefore assumed that in addition to the intracellular chlamydospores there were also free chlamydospores and thin-walled endoconidia in the atmosphere, and that some of these would eventually be deposited on favourable infection courts on new host plants in a cacao field.

Experiments using frass as inoculum

To determine whether the frass was in fact inoculum, inoculation experiments were performed. Diseased trees were eradicated and removed to the laboratory. Frass coils were collected as they were extruded from the insect galleries. To facilitate the application of inoculum this frass was subsequently mixed thoroughly with sterile potato dextrose agar and applied to test plants using the technique described above for inoculation with pure culture. In each case an equal number of controls was treated in the identical manner except that sterile potato dextrose agar was applied to the half-girdles. The results are shown in Table II. The histological picture of these frass-inoculated plants was again similar to that in the plants inoculated with pure culture.

TABLE II
Frass inoculation

Clone	No. of plants	Inoculum	Age of frass (in Days)	Time for first symptoms (in Days)	Time for death (in Days)	Deaths	
						No.	Percentage
ICS 39	5	Frass + potato dextrose agar	5	32	40	3	60
39	5	Sterile potato dextrose agar	—	—	—	0	0
39	5	Frass + potato dextrose agar	1	30	45	4	80
39	5	Sterile potato dextrose agar	—	—	—	0	0

Germination tests on spores in frass

To test the viability of the spores in the frass, germination trials were made. Frass coils were collected from diseased trees removed to the laboratory. The collections were stored and at intervals small quantities were incubated. Several different media were tried and different incubation methods employed at laboratory temperatures (28-30°C.). Germination counts were made by traversing each slide systematically until 200 spores were counted under the low power of the microscope. Several replicates were examined.

The thin-walled endoconidia in fresh frass germinated fairly readily (Fig. 7) both in liquid medium and in a moist atmosphere without free liquid. Counts of over 60% germination in 24 hours were not uncommon in some media. The germination percentage of the thick-walled

stem in 10 wilting trees from different localities in River Estate. Each tree was eradicated, removed to the laboratory and the stem marked off into 25 cm. regions beginning at soil level. Trunk region 1 therefore represents from soil level to 25 cm. up the stem, region 2 from 25 cm. to 50 cm. up the stem, and so on. Girth measurements were taken at soil level. The number of effective borer galleries was recorded. An effective borer gallery was taken as one which could be discerned as an orifice on the surface of the bark and traced into the wood by dissecting away the bark. Orifices of the same diameter (approximately 1 mm.) showing on the surface of wood which was actually exposed in the field were also taken as effective galleries when probing with a seeker confirmed that they were not of a fortuitous nature.

From observation in the field it is known that copious quantities of frass are produced. The most prolific region



FIGURE 7
Germinating thin-walled endoconidium of *C. fimbriata*.

chlamydospores was very different, however. It varied from 0 to 7%, the results apparently depending on age of frass and nature of medium. Fig. 8 shows a germinating chlamydospore. This poor germination rate may be an index of low viability of chlamydospores. It may, on the other hand, have no direct correlation with true chlamydospore viability. The former possibility does not appear to support strongly the hypothesis that the chlamydospores are of high significance in disease transmission, but these germination results may be attributable to a lack of knowledge either of the internal factors in the spore which influence its germination capacity, or of the precise environmental conditions optimal for chlamydospore germination. This problem needs further study.

Inoculum potential

The levels of infestation of diseased trees by the *Xyleborus* borers are high and consequently the quantities of frass produced are also high. Table III shows figures for the density of borer galleries in different regions of the

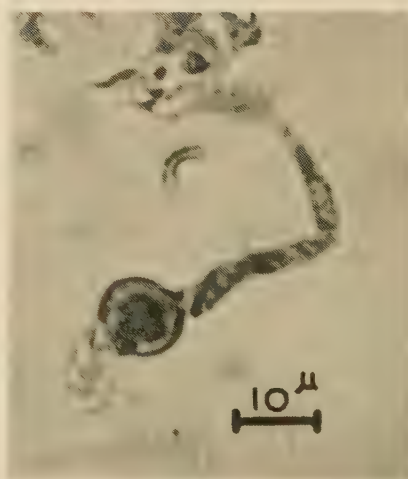


FIGURE 8
Germinating chlamydospore.

Branch showing frass plugs extruded by *Xyleborus* beetles.

TABLE III
Distribution of galleries in cacao stem

Tree number	1	2	3	4	5	6	7	8	9	10
Constitution	ICS I	ICS I	Seedling*	Seedling*	ICS 48	ICS 69	Seedling*	ICS I	ICS I	ICS I
Girth at soil level (cm.)	63	73	48	45	48	53	33	43	45	50
Total number galleries	505	647	670	373	1,165	872	472	293	341	657
Number and percentage of galleries in trunk region	4	0	% 55	% 16	% 151	% 70	% 0	% 0	% 0	% 0
	3	65	20.9	17.0	16.8	97	0	0	0	0
	2	181	26.3	30.8	167	232	146	32	85	208
	1	259	44.6	36.2	651	473	326	261	256	449

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period calculated from the untransformed data was 6.9 mm. The mean and standard deviation of the transformed data ($\log_{10}$) were 0.7927 ± 0.2015 .

The frass plugs collected during the above recordings were stored intact and air-dried in the laboratory for 2 months. The lengths of random samples of these were then measured and their weights taken. The diameter of the plugs was taken as constant as it was found (Iton and Conway, 1961) that *Xyleborus ferrugineus* Fab. and/or *X. corniculatus* Schedl predominated in any infestation and the diameters of their galleries are virtually the same. From this data the weight of unit length of plug was determined (Table IV).

From the mean and the data in Tables IV and V it was estimated that the average gallery in trunk region 1 was liberating some 71,000 chlamydospores per day. The actual number of galleries actively producing frass on each tree was not counted but it was estimated that approximately 40% of those in trunk region 1 was actively extruding frass. From the 10 trees in Table III it was computed that the average number of galleries in trunk region 1 was 359 and that the daily chlamydospore output for that region was approximately 10,250,000. Table III shows that that region contained on the average 60% of the total number of galleries in the stem. Assuming a similar degree of gallery activity and of chlamydospore density

TABLE IV
Relation of weight to length of air-dried frass plugs

Sample No.	Length in mm. of composite samples of frass plugs	Total weight of sample (in mg.)	Mean weight per mm. (in mg.)
1	50	29.4	0.6
2	33	23.6	0.7
3	32	23.3	0.7
4	59	32.2	0.5
5	43	25.5	0.6
	217	134.0	0.62

Samples of plugs were also taken from the air-dried collection, crushed and mixed thoroughly and 1 mg. portions of this powder agitated vigorously in a few ml. of water. The walls of the specimen tubes were then washed thoroughly to remove all particles and the suspensions made up to 10 ml. and centrifuged down. The supernatant liquid in each was pipetted off to leave a constant volume of concentrated suspension. From these remaining suspensions aliquots were removed by pipette for making chlamydospore counts. The frass particles were too large to permit the use of a haemocytometer and therefore counts were made on aliquots mounted on an ordinary microscope slide. The entire area of the preparation was examined. The results are shown in Table V.

in frass at higher levels in the tree than the total daily tree liberation would be approximately 17,000,000 chlamydospores.

No quantitative value of its density at source has been computed for the thin-walled endoconidium as has been done for the chlamydospore. However, the numbers of endoconidia produced are very large and, with germination capacities as high as 60%, it is highly probable that they contribute very heavily to the total inoculum potential.

Discussion

Inoculum source and transmission

From the pattern of spread of disease and the results of spore-trapping experiments at River Estate (Iton, 1959)

TABLE V
Chlamydospore counts

Suspension	Spore count per aliquot	Mean count per aliquot	No. of aliquots per mg. suspension	Mean spore count per mg. frass
1	304	333	25	8,325
2	351			
3	350			
4	325			

and also from the results of the investigations reported in the present paper the following conclusions are drawn:

1. That wind transmission is an important mode of spread of *Ceratostomella* wilt disease in cacao.
2. That chlamydospores and thin-walled endoconidia are the spore forms chiefly produced in the field and are likely to constitute the major part of the inoculum potential.
3. That sporulation does not occur extensively on the external surfaces of the host.
4. That in the field chlamydospores are produced intracellularly in the host.
5. That thin-walled endoconidia are produced only on wood surfaces such as wounds, gallery walls, and on frass heaps.
6. That these two spore forms are well placed for liberation and are actually liberated from the diseased host to the atmosphere in the copious quantities of frass extruded by the boring *Xyleborus* beetles which are invariably present in cacao infected with *C. fimbriata* in the field.
7. That these two spore forms are deposited in suitable infection courts on new hosts. There they germinate to initiate new infections.
8. That the chief infection zone is the axis of the host at or near soil level.
9. That the *Xyleborus* beetles are of primary importance as they provide a highly efficient mechanism whereby infective material is liberated to the wind for transmission.

Evidence for the nature of the inoculum is provided by the production of similar spore forms both in artificially and in naturally infected plants. Information on the source of inoculum is supplied by the similarity in siting of sporulation in these plants.

In wind dispersal of spores the first phase of transmission is liberation from the source of production. Getting the spores away from the surface across the boundary layer of non-turbulent air at the solid-air interface presents a considerable problem to fungi (Gregory, 1952). The present evidence shows that sporulation of *C. fimbriata* occurs chiefly within the host. There would seem, therefore, to be two problems in the liberation of spores of the pathogen. First, that of getting the spores to the surface of the host, and secondly that of getting them away from the surface. Both these problems are very effectively overcome by the mechanism whereby the *Xyleborus* borers extrude spore-laden frass from their galleries in the form of highly friable plugs which project several mm. into the air (Fig. 9).

That the frass is disseminated and deposited has been shown by spore trapping (Iton, 1959) and also by the observation that leaves and trunks of trees surrounding an infected tree are often covered with an obvious coat of the dust.

The infective capacity of the frass is proved by the frass inoculation experiments (Table II). The fact that the incubation period in these experiments was longer than in inoculation using pure cultures (Table I) may be due to the relatively smaller dose of inoculum in the former. It must also be remembered that the frass contains chiefly chlamydospores whereas the pure culture contains chiefly

the high-germinating endoconidia and relatively few chlamydospores. The longer incubation period may therefore be, in part, due to some peculiar germination phenomenon of the chlamydospores. The ages of the frass used in the inoculations were 1 and 5 days, respectively. These periods are quite adequate to permit transport and deposition on other hosts. In trials chlamydospore germination occurred in frass up to 14 days old.

Despite the fact that no wind-borne spores collected away from source have been tested for viability it seems reasonable to assume that they would retain their viability for at least several days. In the field longevity of such spores would depend on their ability to withstand desiccation and insolation. The thick walls of the chlamydospores indicate that they are well equipped to survive such hazards. The thin-walled endoconidia do not appear to be particularly hardy, but in fact the humid, shaded conditions in a cacao field and the proximity of new hosts could hardly be expected to take heavy toll of the spores of this pathogen. Even if they did the large numbers of spores released from each tree would seem to suffice for successful disease transmission.

Studies of the duration and levels of frass production by trees in the field are contemplated, but until these are completed no reliable assessment can be made of the amount of inoculum produced over a long period. Such an assessment would be influenced by a complex of factors including variation in age and density of galleries within the total beetle infestation period of a tree. Iton and Conway (1961) have shown that such variation is considerable. Other influencing factors include degree of colonisation of the tree by the pathogen and also possible variation in excavation activity of the *Xyleborus* beetles which latter Gadd (1949) has reported for the related species *X. formicatus* Eichh. in tea. Further, the cumulative effect of frass production on inoculum potential would depend on factors such as climatic conditions and the longevity of the spores. The attempt at a measure of the inoculum potential in this discussion is therefore aimed at getting some insight into the numbers of infective propagules that are released for wind transmission over a short period within the epidemic. The details of the mechanism of the inoculum potential effect with varying concentrations of spores as discussed by Garrett (1960) are unknown for this disease, but even accepting the laboratory germination percentages as obtaining in the field it would appear that the chances of successful disease transmission are still high in view of the large inoculum potential.

Infection zone

The conclusion that the base of the trunk is the chief infection zone is based on the observation that the wood of wilting or dead plants in the field is in the great majority of cases most intensely discoloured at or near soil level. The observation that in artificially inoculated plants the most discoloration occurs in the inoculation region gives experimental support to this conclusion. According to Malaguti (1958) crotches and natural crevices in the tree provide suitable penetration points for the pathogen. Malaguti (1958) and Naundorf *et al* (1956) also proved that the pathogen was a wound parasite. It appears that the base of the trunk would be a suitable site for infection from wind-borne inoculum as it has many crevices and receives numerous wounds from cultural operations such as cutlassing of weeds. Further, it is probable that fra

washed down the trunk by the run-off water from rain is deposited there. There is also the possibility that the microclimate near ground level may favour infection.

Disease control

In considering disease control through the disruption of the infection chains maintained by wind transmission, the lower trunk deserves particular attention. It not only makes the heaviest contribution to inoculum potential by virtue of having the highest density of borer galleries and, consequently, of frass production, but it is also the chief region through which the pathogen invades the host. However, as the practical aim is disease control, the problem of wind transmission cannot be approached in isolation from other modes of transmission. It would appear that a very effective control method would be the indirect one of controlling the *Xyleborus* borers so as to reduce the inoculum potential available for wind transmission. Moreover, although actual insect transmission has not been proved in Trinidad the borers have been shown to be potential vectors of the disease (Iton, 1960), and Naundorf *et al* (1956) have demonstrated that they are vectors in Colombia. Control by insecticidal applications therefore becomes even more desirable as it would reduce two contributory factors to the propagation of this disease.

At River Estate eradication and burning had been practised from the outset, but in November, 1959, by which time the mortality had risen to about 2%, a comparison of different control methods was begun. The trials included eradication, insecticide spraying, eradication in conjunction with insecticide spraying, and no treatment. No statistically designed experiment could be laid down but the fields to be studied were divided into sections and different treatments were applied to different sections.

Henao (1958) had reported *Xyleborus* borer control in cacao in Colombia by several different insecticides of which 0.25% BHC applied at an interval of one month was the most effective. In addition Fisher and Thompson (1952) reviewed trials made with gamma BHC to protect unbarked logs in Ghana and Malaya. In the former country 0.5% water suspensions gave complete protection for 6 weeks during the rainy season. In the latter 0.65% water suspensions gave complete protection for 4 weeks in dry weather although rainfall later permitted infestation. The same authors also cite reports from French Congo and from warm humid Gulf Coast States in the U.S.A. that adequate protection was provided by BHC against borers of the superfamily Scolytoidea. Gamma BHC was therefore used at River Estate. A 0.65% aqueous suspension was mist-blown on to the tree trunks at intervals of two weeks in fields with high disease incidence and of one month in fields with lower disease incidence. Each trunk was sprayed just enough to wet the surface but avoid run off.

Because of certain factors inherent in the disease distribution pattern and also because of the different levels of disease incidence a categorical evaluation of the efficacy of the several control methods tried is difficult, but it is clear that BHC, as applied at River Estate, provides no effective control. As mentioned above the mortality rose from 2% in November, 1959, to 5% by the end of 1960. Further, the data in Table III, which were collected in January, 1961, and borer gallery analysis, also done in 1961 (Iton and Conway, 1961), together show that borer invasion of the stem still continued despite the frequent gamma BHC applications. In January, 1961, gammexane

was replaced by dieldrin and the spraying cycle was maintained. Intensive tests of the efficacy of different insecticides may be profitable. This would seem necessary because experience at River Estate and other reports in the literature indicate that the success of a given insecticide is not necessarily universal. Another example is dieldrin. This has been successful against *X. formicatus* Eichh. in tea in Ceylon (Austin, 1956; Judenko, 1960), but gives no adequate control against *X. morstatti* Hag. in cacao in Ghana (Anon., 1960). However, from information now available of some of the habits of the borers (Iton and Conway, 1961), it is clear that even if an insecticide were found to prevent stem infestation the whole problem of disease transmission may not be solved as the beetles very actively invade the tree below soil level and it is not certain that they are not direct vectors of the disease. These studies on insect activity have not been exhaustive and the need for more intensive investigations into this aspect of the complex situation is abundantly clear.

Systemic applications of insecticides to the tree may be worth while, but even this may not solve the entire problem. Killing the borers on or in the host will prevent frass extrusion but will not necessarily prevent infection from spores which these vectors may carry, as these spores will remain in contact with the host. However, Bowman and Casida (1958) have shown that there are systemics which are readily translocated in cacao and leave little or no harmful residues in the beans. Further, Al-Azawi and Norris (1959) have used one of these systemics with some success in preventing transmission of *Ceratocystis ulmi* (Buis.) Moreau by *Scolytus multistriatus* (Marsh.) which is one of the vectors of Dutch elm disease and is related to *Xyleborus* spp. Parallel trials in controlling *Ceratostomella* wilt in cacao are indicated.

An alternative method which may effect control would appear to be the use of fungicides in conjunction with contact insecticides. Total insect control would seem unlikely and, to prevent the successful penetration of germ tubes developed from spores in wind-borne frass deposited on host plants, prophylactic fungicidal applications to the tree trunk may be useful. This is probably the mechanism which operated in a preliminary spraying trial reported by Oechsli (1957) for an estate in Ecuador. 3% cuprous oxide mixed with 3% DDT was applied heavily to trunk and main branches on 3 occasions over a year and appeared to give good control. He also points out that in Mexico routine commercial spraying with combined insecticide and fungicide indicates that control can probably be effective. It may be practicable to develop a technique for estimating the rate of frass production in the field. Studies on possible seasonal variation in inoculum potential may then contribute to a satisfactory and economic design of protective spraying cycles. Fungicides should also offer the additional advantage of protection against disease transmitted by spores which may originate on cut surfaces and be blown on to new hosts.

Wounding should be avoided as far as possible as it increases the possibility of the formation of spores in positions suitable for wind dissemination. It also increases the area available for initiation of infection in healthy trees. Where pruning is done the freshly exposed surfaces should be treated with fungicides.

The need for field sanitation is obvious. Diseased trees should not be cut into pieces and left in the field because exposure of wood surfaces induces prolific sporulation.

Eradication of the entire tree, followed by immediate burning of all parts should be practised. If this is done before many borers invade the tree it would prevent the liberation of much frass for wind dissemination.

Cultural control may also contribute to reducing the incidence of this disease. In Ghana (Anon., 1960) it has been found that *X. morstatti* Hag. attacks fewer cacao plants in bare soil and in mulched plots than in plots with growing cover. The trees in that country are not infected, but Iton and Conway (1961) have postulated that diseased trees do attract the borers. It would therefore be interesting to test cultural control in these latter conditions. Destruction of alternative breeding places of the beetles would seem a necessary supplement to cultural control, but one which is likely to be very difficult.

Resistance to *Ceratostomella* would probably be the best method of control. This has been suggested before by several South American workers and, as Desrosiers (1956) points out, the apparently high susceptibility of Criollo and resistance of Trinitario and Forastero suggests that cacao developed for resistance to Witches' broom will probably be resistant also to *Ceratostomella* wilt.

Summary

The spore forms of *C. fimbriata* in culture are studied. The sporulation pattern in artificially inoculated plants is compared with that in diseased plants in the field. It is concluded that sporulation does not occur extensively on the external surface of the host and that the chlamydospores and thin-walled endoconidia which are respectively produced intracellularly in the wood and on wood surfaces such as the *Xyleborus* galleries and frass heaps constitute the major portion of the inoculum.

The great importance of the *Xyleborus* borers in promoting spore liberation is discussed.

It is established that the frass extruded by *Xyleborus* borers which infest the diseased trees is effective inoculum and is wind-borne. The chlamydospore content of this frass is determined numerically. The germination capacity of these spores and of the thin-walled endoconidia, also present in the frass, is studied and confirmation is obtained for the foregoing conclusions.

It is shown that the region of the host in which infection is chiefly initiated and also from which most wind-borne inoculum originates is the lower trunk and its significance in disease control is indicated.

Control measures are discussed and indications given of different approaches which may be made to this problem in the future.

Acknowledgments

The author wishes to thank Dr. F. W. Cope, of the Regional Research Centre, for his help and advice in the processing of the numerical data and for his interest in all stages of the investigations. Thanks are also due to the Commonwealth Mycological Institute for identification of the fungi.

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Studies on a wilt disease of cacao at River Estate

III. Some aspects of the biology and habits of *Xyleborus* spp. and their relation to disease transmission

by

E. F. Iton and G. R. Conway

Introduction

WILT disease in cacao caused by *Ceratostomella fimbriata* (Ell. & Hals.) Elliot has been reported in most of the cacao growing countries of South America and from Costa Rica and Mexico in Central America. In each case there is a close association between the disease and beetles of the superfamily Scolytoidea, chiefly of the genus *Xyleborus* Eichh. (Scolytidae). Consequently the condition has been frequently referred to as the "*Ceratostomella-Xyleborus* complex." Almost invariably a significant role in the transmission of the disease has been ascribed to the beetles. Idrobo (1958) briefly reviews the literature regarding this complex in Colombia and cites experimental evidence which shows that the borers are vectors of the disease. Desrosiers (1958) states that in Ecuador observations lead to a similar conclusion. In Trinidad proof that the beetles transport the pathogen from host to host in the field has not yet been obtained, but Iton (1960) has demonstrated the presence of viable chlamydospores in the rectal regions of the alimentary tract in a large number of beetles extracted from borings in *Ceratostomella*-infected cacao (Fig. 1).

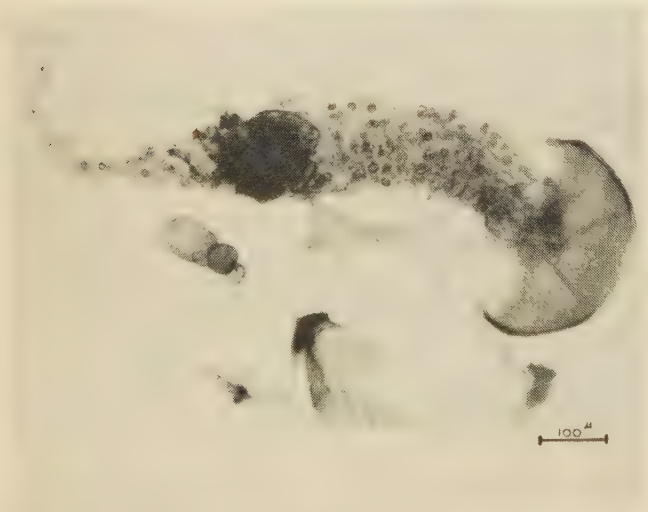


FIGURE 1

Viable chlamydospores of *C. fimbriata* in rectum of *X. ferrugineus*.

This, in conjunction with the observation that the borers are frequently found on the wing, is taken as circumstantial evidence that they can act as vectors. Further, they have an important role in promoting wind transmission and this has been discussed by Iton (1961).

Only in Venezuela have experiments suggested that the insects do not play a significant part in spreading this disease (Malaguti, 1958). But even there it is conceded that they may be casual vectors of some importance.

Despite this weight of evidence for the importance of the beetles in transmission of the disease in one way or another, little seems to be known of their biology and habits. Iton (1960) stated that the practicability of controlling the disease through control of the insects could not be assessed until knowledge of their habits had been obtained. The need for knowledge of this kind has become even clearer as insecticides which have been reported to control the beetles elsewhere (Henao, 1958) have failed to do so at River Estate. Investigations have therefore been initiated to fulfil this need and these are reported in this paper.

Borer species

Iton (1960) listed six species of *Xyleborus* which had been obtained from *Ceratostomella*-attacked cacao in Trinidad. One of these (*X. sp. nov.*) has since been described by Schedl (1960) and named *X. trinidadensis*, and *X. confusus* Eichh. is a synonym of *X. ferrugineus* Fab. (Wood, 1957). Three more species of *Xyleborus* and one of the genus *Platypus* have also been collected from this habitat. The corrected list thus contains nine species:—

Scolytidae

Xyleborus corniculatulus Schedl*

X. ferrugineus Fab. (= *X. confusus* Eichh. = *X. fuscatus* Eichh.)

X. hirtellus Schedl† (= *X. theobromae* Hopkins)

X. posticus Eichh.

X. spinulosus Blandf.

X. torquatus Eichh. (= *X. volvulus* (Fab.))

X. trinidadensis Schedl

X. varians Fab.

Platypodidae

Platypus pulicarius Chap.

Only the more important synonyms are cited. Of the species listed *X. ferrugineus* Fab. and *X. corniculatulus* Schedl are the most frequently encountered, either one or both forming the major element in any infestation. Several of the other species appear to attack cacao trees only when infestation by *X. ferrugineus* or *X. corniculatulus* or both is already advanced. The other species are therefore

* This should not be confused with a closely related species *X. corniculatus* Schedl which has been reported as of importance in *Ceratostomella*-attacked cacao in Colombia (Steinhausen, 1956).

† In Iton (1960) this was misspelt as *X. hirtella* Schedl.

probably of secondary importance in promoting the transmission of the disease.

The beetles make borings, more commonly referred to as galleries, within the wood of the host tree and appear to live in these for all but a short period of the life cycle. Consequently they cannot be observed directly under natural conditions and it is only possible to deduce aspects of their biology from the structure, distribution and contents of galleries which have been dissected out.

Gallery structure and life cycle

Numerous galleries of *X. ferrugineus* were examined in some detail. The adult female usually begins by boring a straight tunnel about one millimetre in diameter, extending into the wood at right angles to the bark. In this simple, unbranched form, containing only one adult usually and no other stages in the life cycle, the tunnel is termed an "incipient gallery." The beetle then constructs a branch at a point, which in the galleries examined varied from 5-30 mm. from the entrance hole, and at the end of this branch tunnel the first eggs are usually laid. They are white and ellipsoidal to ovoid in shape. They are to be found in clusters of up to ten but it is not known whether they are laid simultaneously or singly over a period of time. Branching continues, producing an irregularly dichotomous system lying approximately in one horizontal plane. More than one adult is often to be found in a single gallery system, but the contribution of each of these to the construction of the system is not known. The larvae are whitish with a dark-brown chitinised head capsule and are apodous, roughly cylindrical and curved. They live freely in the tunnels, apparently without excavating the wood to any extent, and pupate there after passing through several instars. The adults on emerging from the pupae go through a teneral phase before leaving the parent gallery. During this the cuticle hardens and the colour changes from a light yellow to a dark reddish-brown. It would appear that several broods are reared in a gallery system, the adults laying the eggs of each brood at the end of a newly formed branch which is subsequently extended. Thus a mature gallery system contains all or most of the stages in the life cycle distributed apparently at random throughout it.

Tunnels from different systems do not join with one another. The beetles are apparently able to detect the presence of other tunnels in the wood and will cease boring just before making contact with them. There is, however, evidence that tunnels running vertically in the tree may be produced, from which are then constructed, at various levels, dichotomously branched systems with tunnels to the exterior. Neither the frequency with which such multiple gallery systems occur nor the extent to which their horizontal tunnels constitute exit holes is known.

At any given level the galleries are not uniformly distributed in the wood but tend to be concentrated in certain sectors of the stem, branch or root. They exhibit various stages of fungal attack and degrees of staining. In general the oldest galleries are darkest stained while the youngest are usually clean and fresh looking with no apparent sign of fungal attack. This staining is not necessarily due to *C. fimbriata* as other fungi, notably *Calonectria rigidiuscula* (Berk. & Br.) Sacc. (synonym *Fusarium decemcellulare*) are present. Mites are to be found in abundance in many of the abandoned galleries and to a lesser extent in some of

the younger galleries containing stages in the life cycle of the beetle.

A few galleries of *X. corniculatus* were examined. These are of slightly less diameter than those of *X. ferrugineus*, reflecting the smaller size of the former species. They show the same general dichotomous pattern of development without any striking dissimilarities, although the interval between branching seems slightly less.

Distribution of galleries

Observations in the field show that the highest incidence of borer galleries in the majority of wilted trees occurs in the base of the trunk and the collar. Although in some trees infestation may be confined to one or more of the upper branches, this condition is much less frequent and is associated with death of the branches only (Iton, 1959). Wallenius (1958) and Idrobo (1958), in Ecuador and Colombia, respectively, both reported high incidence of galleries at the base of the trunk, but neither recorded infestation in the roots such as occurs in Trinidad (Fig. 2).



FIGURE 2
Ceratostomella-attacked cacao root showing frass plugs extruded by *Xyleborus* spp.

A survey of the entire axis of the tree was undertaken to determine numerically the distribution of the galleries.

Preliminary observations had shown that in the majority of wilting trees few borings occurred above 100 cm. from soil level. Ten diseased trees at advanced stages of wilting were therefore eradicated from different localities in River Estate and the stems beyond 100 cm. above soil level

discarded. The roots were washed free of soil and the tree axis marked off into 25 cm. regions beginning at soil level. Trunk region 1 therefore represents from soil level to 25 cm. up the stem, region 2 from 25 cm. to 50 cm. up the stem, and so on. Regions were similarly delimited on the major roots. Girth measurements were taken in the middle of each region. The number of galleries was counted, the criteria used for recording a gallery being the same as defined in Iton (1961). The results are shown in Table I.

Fig. 3 further illustrates this pattern by showing the details of distribution of galleries in a single tree. The number of galleries per 5 mm. length is shown and a ground level zone delimited. This zone is defined as including soil level and the layer of litter above it.

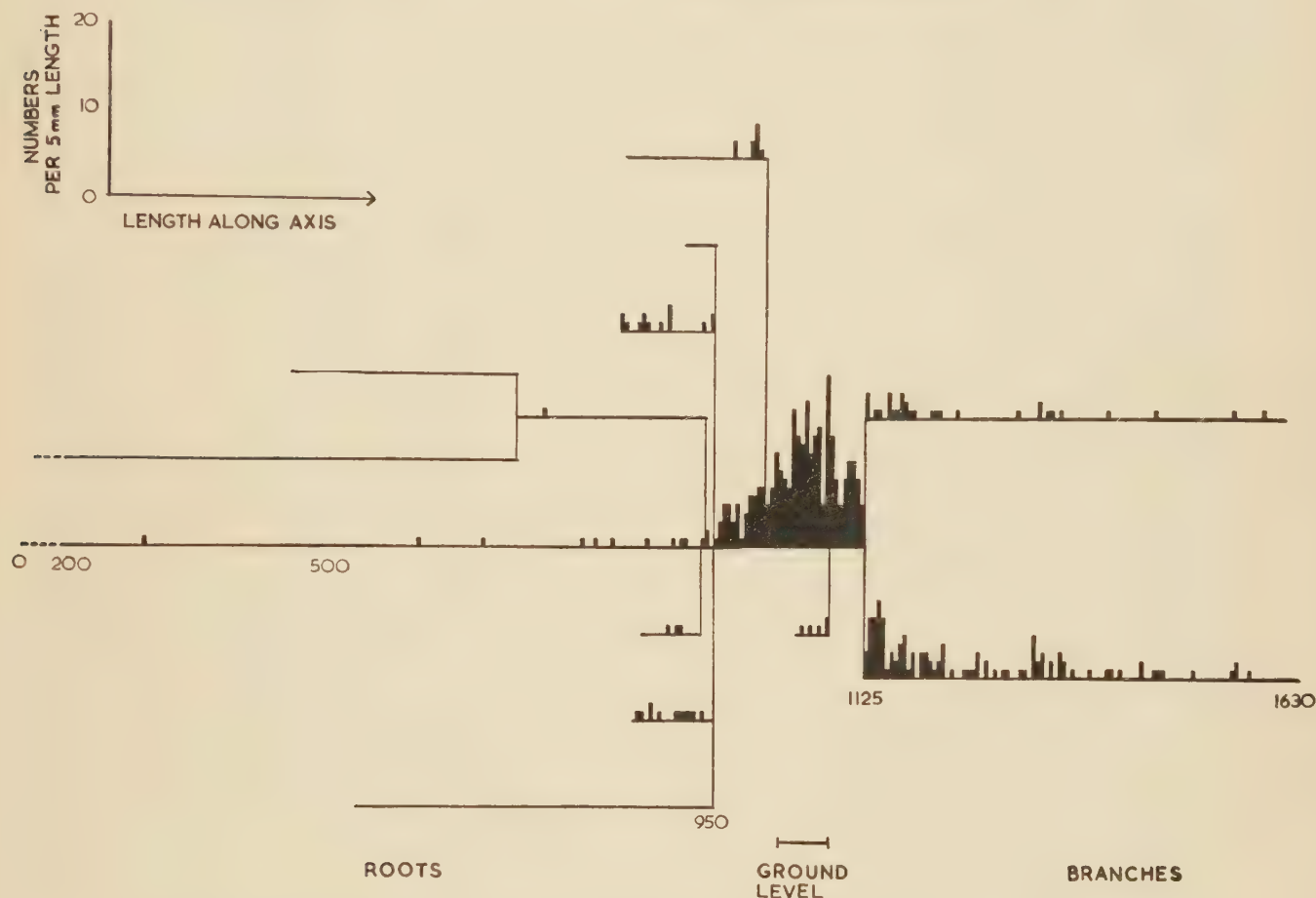


FIGURE 3
Number of *Xyleborus* galleries per 5 mm. length in a single *Ceratostomella*-wilted cacao tree. Length along axis measured in mm., taking the tip of the deepest penetrating root as origin.

Analysis of population

Further study of the insect population in the galleries has provided data which enable a partial interpretation of the pattern of distribution described above. The technique employed was simple but inevitably laborious and thus only a single tree could be examined in the time available. A recently killed tree with a representative but not too high gallery density was therefore selected. This was sawn into logs which were placed in an oven at 130°C. to kill the insects *in situ* and the logs were then cut with a band saw into slices of approximately 5 mm.

thickness. It was estimated that the saw removed about 1 mm. between slices. In the laboratory the thickness of each slice was measured and the number of holes in the bark recorded. The slices were then broken up and examined, the structure and contents of the galleries being noted.

The histograms in Figs. 4 and 5 represent the frequency per slice of incipient galleries, eggs, larvae, pupae, teneral and mature adults and galleries. Data from slices of separate roots and branches have been amalgamated as if the tree formed a single axis. As the slices vary in thickness this leads to some inaccuracy in matching slices from the same level. However, the majority of slices are of 6 mm. thickness (inclusive of the 1 mm. removed by the saw) and it is only in the upper regions of the branches that any appreciable discrepancy occurs.

Table II gives the total numbers of galleries, incipient galleries and stages in the life cycle for the whole tree and also the totals and proportions for the ground level zone.

Study of Table II and of the histograms in Figs. 4 and 5 quite clearly reveals the pattern of distribution. Although incipient galleries are to be found at all levels of the tree they form the majority of the galleries at the upper and lower limits of infestation. Eggs occur between rather narrower limits and have two peaks of abundance, one above and one below ground level. Larvae and pupae have similar but less extensive distributions, while the

TABLE I
Distribution of *Xyleborus* galleries in cacao

Type of Tree	Cutting			Seedling			Seedling			Cutting		
Total Number of Galleries	839			1,141			1,241			611		
	Girth in cm.	No. of galleries	Per-centage	Girth in cm.	No. of galleries	Per-centage	Girth in cm.	No. of galleries	Per-centage	Girth in cm.	No. of galleries	Per-centage
Trunk Region	—	0	0.0	—	0	0.0	33	55	4.4	33	59	9.7
	30	65	7.5	—	0	0.0	40	140	11.3	35	64	10.5
	38	181	21.6	38	162	14.0	38	176	14.3	38	115	18.9
	63	259	31.0	80	485	43.0	43	299	24.0	43	135	22.0
Soil Level												
Root Region	63	276	33.0	65	483	42.0	53	535	43.1	45	217	35.5
	30	57	6.8	25	11	1.0	38	36	2.9	38	21	3.4
	20	1	0.1	—	—	—	—	—	—	—	—	—

Type of Tree	Cutting			Seedling			Cutting			Cutting		
Total Number of Galleries	1,322			809			565			710		
	Girth in cm.	No. of galleries	Per-centage	Girth in cm.	No. of galleries	Per-centage	Girth in cm.	No. of galleries	Per-centage	Girth in cm.	No. of galleries	Per-centage
Trunk Region	35	70	5.0	—	0	0.0	—	0	0.0	—	0	0.0
	35	97	7.0	—	0	0.0	—	0	0.0	—	0	0.0
	40	232	18.0	33	146	18.0	33	32	5.6	30	85	11.9
	55	473	36.0	40	326	40.3	43	261	46.0	38	256	36.1
Soil Level												
Root Region	50	433	32.7	23	337	41.7	43	234	41.4	50	298	42.0
	23	17	1.3	—	—	—	13	38	7.0	20	71	10.0
	—	—	—	—	—	—	—	—	—	—	—	—

teneral adults show a narrow distribution with a single pronounced peak in the ground level zone. The mature adults occur at all levels of the infestation and in the majority of galleries.

Discussion

These findings show that the population is developmentally most advanced in the galleries at ground level and is progressively less so in the galleries above and below this level. From this conclusion one of two

inferences can be drawn. Either that conditions for the development of the insect are more suitable at ground level than at higher and lower regions, so that the population, although of uniform age, has developed at different rates at different levels; or that the population at ground level represents a longer-standing infestation.

If the first inference is accepted then the majority of the galleries must have been constructed at the same time. Careful examination of the pattern of distribution as shown

TABLE II
Analysis of *Xyleborus* population in a *Ceratostomella*-wilted cacao tree

	Galleries	Incipient Galleries	Eggs	Larvae	Pupae	Teneral Adults	Mature Adults
Total for whole tree	488	42	262	452	193	394†	574*
Ground level	152	1	14	48	50	191	216
Per cent of total at ground level	31.14%	2.38%	5.34%	10.62%	25.91%	48.48%	37.63%

* This figure is made up as follows : *X. ferrugineus*, 545 females, 16 males.
X. corniculatulus, 2 females.
X. posticus, 7 females.
Platypus pulicarius 4.

† All the teneral adults were *X. ferrugineus*. The figure is composed of 381 females and 13 males.

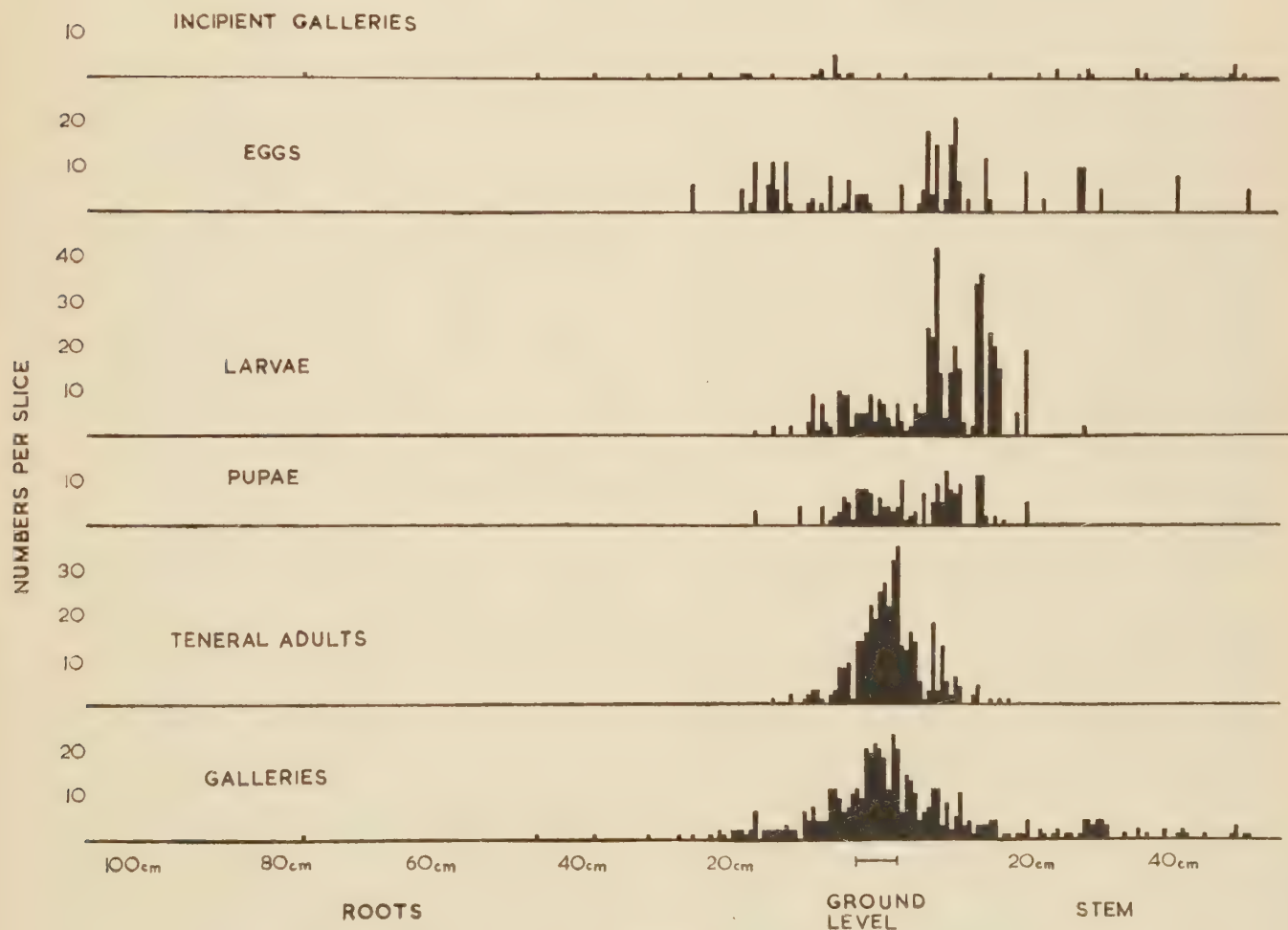


FIGURE 4

Number of *Xyleborus* galleries, teneral adults, pupae, larvae, eggs and incipient galleries for a single *Ceratostomella*-wilted cacao tree.

in Fig. 4 suggests, however, that this is unlikely. First, marked differences in the development of the population exist over a very short distance above and below ground level. This would seem to involve a drop in suitability too marked to be accounted for by differences in the environment within the plant body. Secondly, as distance from ground level increases, incipient galleries, evidently newly formed, contribute a progressively higher proportion to the total number of galleries. It is therefore clear that regions furthest removed from ground level are the most recently colonised.

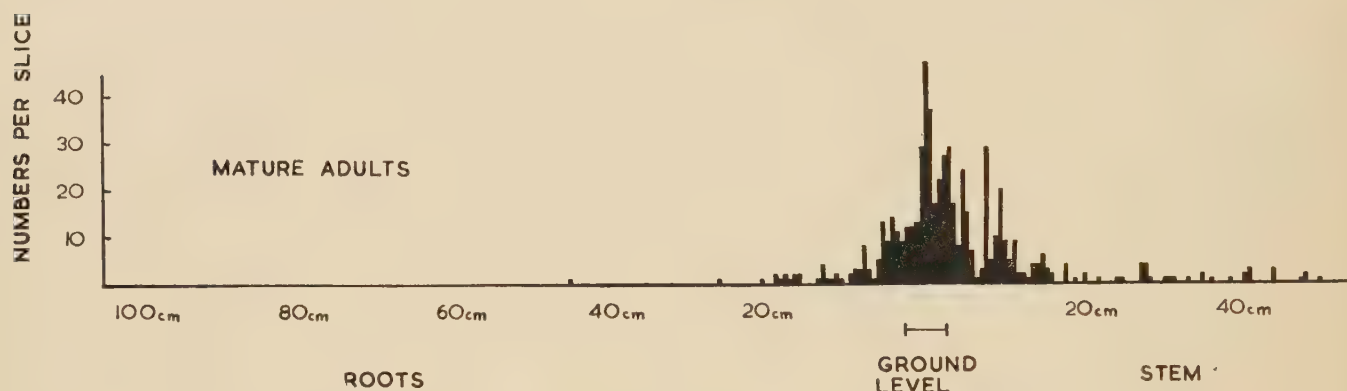


FIGURE 5
Number of mature *Xyleborus* adults for a single *Ceratostomella*-wilted cacao tree.

The second inference thus appears to be more in accord with the distribution described. It can be seen that at ground level the population is composed mainly of the late stages in the life cycle, the major proportion being teneral adults. Above and below this zone the population includes progressively earlier stages in the life cycle, with the major proportion at any given level being composed of stages intermediate between the earliest and latest stages present. At the limits of infestation only the very early stages are to be found. This pattern can be explained by postulating that initially the ground level region is most attractive to the beetles but that in time the attractiveness declines at this level and moves progressively to higher and lower regions of the tree. Thus any given level will pass through a cycle of attractiveness, rising to a maximum and declining again. This will be reflected at any given time by the relative proportions of new galleries and of stages of the life cycle at this level.

The cause of attractiveness is not known with certainty, but there are indications as to its nature. The literature shows an increasing inclination to the view that insects of this and related groups can attack apparently healthy trees (Fisher and Thompson, 1952; Anon., 1958). If this is the case with cacao then it may be that the ground level zone of the healthy tree provides the most accessible site for boring. Schedl (1957) has suggested that the beetles spend a period of diapause in the ground litter. It is possible that young adults, on leaving the parent galleries, migrate to the litter layer and from there bore directly at that level into cacao trees. Then as the density of galleries increases, beetles arriving later are forced to move up and down the tree in search of less heavily infested regions. Attractiveness in this case may be largely determined by accessibility of site for boring and the density of existing galleries. This hypothesis assumes that infestation precedes infection.

The latter could then be accounted for either by vector transmission or by wind-borne inoculum. However, it is more generally accepted that the beetles attack diseased or weakened trees. It is thus possible that infection by wind-borne inoculum precedes infestation. Iton (1961) has shown that in the majority of cases infection is initiated at ground level and spreads up and down the tree. This suggests that attractiveness may be caused by the effect of the disease on the host tissues.

While these hypotheses for attractiveness are not mutually exclusive, the weight of evidence seems greater for the

latter. First, the relation of actual numbers of galleries to girth of tree (Table I) gives no indication that the ground level zone reaches a saturation point of gallery density before exploitation of higher and lower regions by the beetles begins. Some of the smaller trunks carry much higher gallery densities near ground level than do trunks of larger girth. Secondly, as has been mentioned above from laboratory examinations, galleries tend to be confined to one sector and in the field it is not uncommon to observe cacao trees heavily infested on one side only. If the beetles are attracted to the trunk at ground level because of its proximity to the litter layer infestation would tend to be uniformly distributed around the trunk. However, internal examination of asymmetrically infested trees reveals that although infection is advanced in the heavily infested sector, the wood in the uninfested sector is free of infection. Thirdly, infestation does not necessarily occur at ground level and may be confined to the upper branches as described above. These data and observations indicate (1) that proximity to the litter layer is not the cause of attractiveness and (2) that shortage of available sites for boring in the ground-level zone is not critical in determining the outward flow of infestation. On the other hand they do suggest that attractiveness is a function of infection and that infection precedes infestation.

The findings discussed in this paper shed some light on the intimate association of the *Xyleborus* spp. in Trinidad with *C. fimbriata*. The evidence adduced favours the view that infection precedes infestation and consequently implies that wind transmission is of greater importance than vector transmission of the disease. However, as the beetles are very important in promoting wind transmission (Iton, 1961), further study of their habits and development of effective control measures remain critical to the control of the disease.

Investigations are to be initiated into the flight activity of the insects by employing trapping experiments, supplemented, if possible, by labelling insects with radio-active isotopes. Information thus gathered should contribute to the designing of a successful programme for timing and placement of insecticide applications. Further studies on the importance of alternative hosts, *e.g.* *Erythrina* spp. (Iton, 1959) as breeding places of the beetles are also to be made.

Summary

1. Evidence for the importance of beetles of the genus *Xyleborus* (Coleoptera : Scolytidae) in the transmission of *Ceratostomella* wilt disease of cacao is referred to.

2. Species of the families *Scolytidae* and *Platypodidae* which have been found associated with diseased trees in Trinidad are listed. *Xyleborus ferrugineus* Fab. and *X. corniculatulus* Schedl are regarded as the most important species.

3. The structure, contents and mode of formation of the galleries of *X. ferrugineus* is described and those of *X. corniculatulus* are briefly mentioned.

4. The distribution of the galleries in infected cacao trees is investigated and it is shown that they are to be found in the stem, roots and branches but are mainly concentrated at ground level.

5. For a single tree a pattern of population in the galleries is described which suggests that initial infestation is at ground level.

6. Hypotheses to account for the site of initial infestation and for the distribution of galleries are put forward.

7. Indications are given of lines of future research.

Acknowledgments

The authors are deeply indebted to Mr. M. H. Breese, of the Regional Research Centre for his invaluable help and advice. Thanks are also due to Dr. K. E. Schedl, of Lienz, Austria, for identification of the beetles, and to the Commonwealth Mycological Institute for identification of the fungi. The work was carried out while one of the authors (G.R.C.) was in receipt of a Colonial Office Probationership. A part is included in a thesis submitted in part fulfilment of the requirements for the Diploma of Tropical Agriculture (Trinidad).

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Root aeration and the growth of cacao seedlings

by
D. Walmsley

HARDY (1942) stated that the decline of cocoa yields in Trinidad may be mainly attributed to lack of adequate aeration in the root zone. It is therefore desirable to ascertain just what constitutes adequate aeration for the cocoa plant before further studies in the field can confirm this statement. With this object in mind, cocoa seedlings have been grown under controlled experimental conditions where all other factors in the root environment were kept as constant as possible so that variations in growth with known aeration gas compositions could be clearly demonstrated.

A nutrient solution of known constant composition, saturated with a gas mixture of known composition was used as the growth medium since uniform conditions are more easily obtained in this system than in soil. The apparatus used necessitated the use of very young plants, so before the results obtained in these experiments may be applied to cocoa trees in the field one must assume that the growth of these young roots is very similar to the growth of new roots in established cocoa trees.

Apparatus and methods

Tall, 1-litre, glass cylinders were used as culture vessels. They were made light-tight by a covering of black polythene sheeting which could be removed for observations. This prevented algal growth.

The seedlings were grown in half strength Long Ashton solution (Hewitt, 1952). This solution was supplied continuously to the culture vessels by a constant head gravity feed so that the nutrient solution surrounding the roots was of constant composition.

The aerating gas was mixed to the desired composition using previously calibrated flow meters. It was passed continuously through the culture solution at a total rate of 30 litres per hour. The nitrogen gas supplied by the manufacturers could contain about 1% oxygen but this would not significantly affect the results of this experiment.

The culture vessels were contained in a glass box thermostatically controlled at $30 \pm 2^\circ\text{C}$. The whole apparatus was set up in the open air but shielded from direct sunlight.

The testas were removed from about 50 ICS 95 seeds which were then allowed to germinate over water in a covered dish. After three days 12 seedlings having radicles of approximately the same length were selected and transferred to the culture vessels.

Daily records of the root length (measured from the scar on the hypocotyl) were made. After twenty days' growth fresh and dry weights of the roots and shoots of the seedlings were taken and then these were subjected to analysis for nitrogen and phosphorus. Mean dry weights and rates of root elongation were determined.

This procedure was repeated for sets of 12 plants using different aerating gas compositions. Keeping the oxygen constant at 21% the carbon dioxide content was varied over the range 0.03% (air) to 25%, and keeping the carbon dioxide constant at $2\frac{1}{2}\%$ the oxygen was varied over the range 2-30%. Nitrogen was used to make up the mixture to 100%.

Results and discussion

(a) Effect on growth of roots and shoots

Fig. 1 shows the rate of root extension (in cms./day) for different concentrations of oxygen and carbon dioxide in the rooting medium. From these graphs it will be seen that when there is a normal supply of oxygen (21%) the maximum rate of tap root elongation is obtained with $2\frac{1}{2}\%$ of carbon dioxide in the aerating gas. It is not until the carbon dioxide content exceeds 10% that the growth rate is reduced below that in the air.

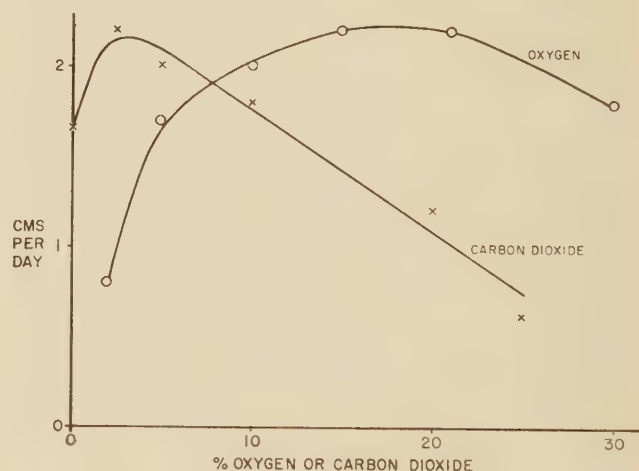


FIGURE 1

The rate of cocoa root elongation with different aeration gas compositions.

o oxygen varied; carbon dioxide $2\frac{1}{2}$ per cent.
x carbon dioxide varied; oxygen 21 per cent.

If the carbon dioxide is maintained at this optimal value of $2\frac{1}{2}\%$, then the oxygen content of the aerating gas may be reduced to 5% before the root growth rate falls below that in air. The maximum rate of root extension, however, is obtained at oxygen contents of 15-21%.

Vine (1940) has suggested, from his measurements of cocoa root elongation, that 9% oxygen in the rooting medium is the lower limit for satisfactory root activity. Bearing in mind that no carbon dioxide was added to the mixture, this conclusion compares favourably with the present work. Fig. 1 shows that below 10% oxygen the

growth rate falls off quite rapidly whereas from 21% to 10% oxygen there is only a small change.

These observations may be compared with those of Leonard and Pinckard (1946) who found that, for cotton root elongation, the optimum carbon dioxide content in the aerating gas was 0-15% when 21% of oxygen was also present. With 10% of carbon dioxide in the mixture they reported the optimum oxygen content as $7\frac{1}{2}$ -21%. The greatest rate of elongation occurred at 21% oxygen with 10% carbon dioxide. It would appear that the root elongation of cotton and cocoa is affected similarly by changes in aeration conditions except that carbon dioxide has less influence on cotton. Ranson and Parija (1955) have shown that for wheat, barley, marrow, buck-wheat and broad-bean the maximum daily rates of growth and final lengths of the roots decreased with each decrease in oxygen concentration below that of air. These decreases are more pronounced below 10% oxygen. Leyton and Rousseau (1958) have also found that the root extension rate of spruce and pine species is decreased at all oxygen levels below that in air but more markedly so below 10%. The root extension rate of citrus seedlings growing in sand culture was reduced to one half that in air by lowering the oxygen to 5-8% with 1-2% carbon dioxide present (Girton, 1927). No carbon dioxide was present in the gas mixtures used in the investigations by Ranson and Parija and by Leyton and Rousseau so a direct comparison with cocoa is not easy to make, but it would seem that cocoa is at least as tolerant of decreased oxygen conditions as any of the plants mentioned.

A more useful measure of plant growth is the dry weight of the tissue concerned. Fig. 2 shows the dry weight of

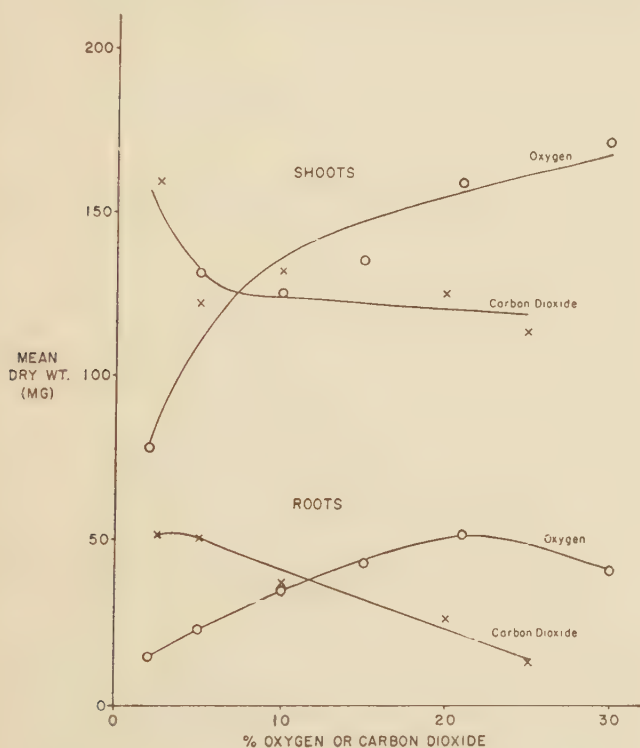


FIGURE 2

The changes in the mean dry weight of cocoa roots and shoots as the aerating gas composition is varied.

- oxygen varied; carbon dioxide $2\frac{1}{2}$ per cent.
- x carbon dioxide varied; oxygen 21 per cent.

the root and shoot of the 20-day-old seedlings at various compositions of the aerating gas. These graphs show that a mixture with 21% oxygen and $2\frac{1}{2}$ % carbon dioxide gives the maximum dry weight of root. Mixtures with more carbon dioxide or less oxygen than this result in decreased root growth. This finding is not in complete agreement with the conclusions drawn from the root elongation rate measurements which show no indication of a decreased rate of growth until the oxygen content falls below 15% and there is very little change at 10%.

Similar observations have been reported from work on apple seedlings by Boynton *et al.* (1938). These authors concluded that normal growth of the existing root tips continues down to 10% oxygen but that higher concentrations (above 12%) are needed for the initiation of new roots from the existing root system. Further investigations (Boynton and Compton, 1943) showed that a decrease in oxygen content from 21% to 15% causes a decrease both in the root weight and the number of new roots formed.

Shoot growth (Fig. 2) also shows a marked dependence on the aerating gas composition. Decreasing the oxygen below 21% results in a corresponding decrease in top growth but, unlike root growth, an increase in oxygen up to 30% produces an increase in shoot weight. An increase in carbon dioxide from $2\frac{1}{2}$ % to 5% results in much lower shoot growth which shows, rather surprisingly, that the shoot is more sensitive to carbon dioxide in the rooting medium than is the root. Ranson and Parija (1955) have stated that on a basis of fresh weight the growth of wheat, rice and broad-bean shoots is increased at oxygen concentrations lower than 21%. This is contrary to the findings (based on dry weight) of this investigation on cocoa and also to those of Taylor (1942) who worked with rice and wheat.

(b) Effect on nitrogen and phosphorus content of root and shoot

Root nitrogen is not greatly affected until the carbon dioxide concentration exceeds 20% or the oxygen falls below 5%. On the other hand root phosphorus is decreased to a marked extent as the oxygen is lowered below 10%. As the carbon dioxide is increased from $2\frac{1}{2}$ % to 10% there is also a decrease in root phosphorus but the effect here is not so great (Figs. 3, 4, 5, 6).

No noticeable change occurs in the nitrogen and phosphorus content of the cocoa shoot as the aerating gas

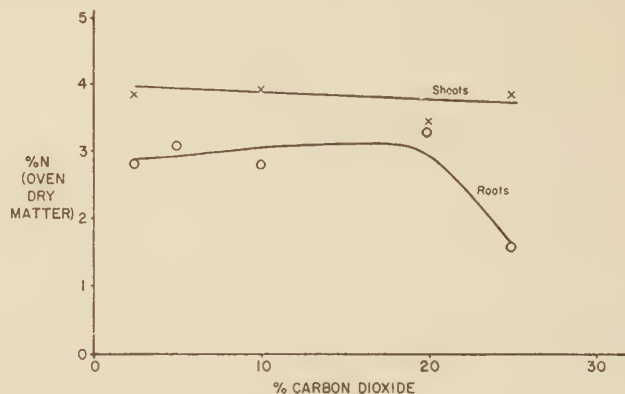


FIGURE 3

The changes in the percentage of nitrogen in the roots and shoots of 20-day-old cocoa seedlings as the carbon dioxide content of the aerating gas is varied. Oxygen held constant at 21 per cent.

composition is varied. At this early stage in the growth of the seedling the cotyledons are still present and this may mean that the shoot does not reflect to any great extent the variations in nutrient uptake by the roots.

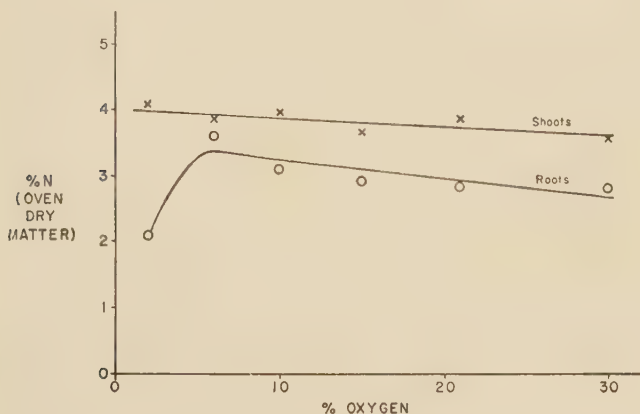


FIGURE 4

The changes in the percentage of nitrogen in the roots and shoots of 20-day-old cocoa seedlings as the oxygen content of the aerating gas is varied. Carbon dioxide held constant at $2\frac{1}{2}$ per cent.

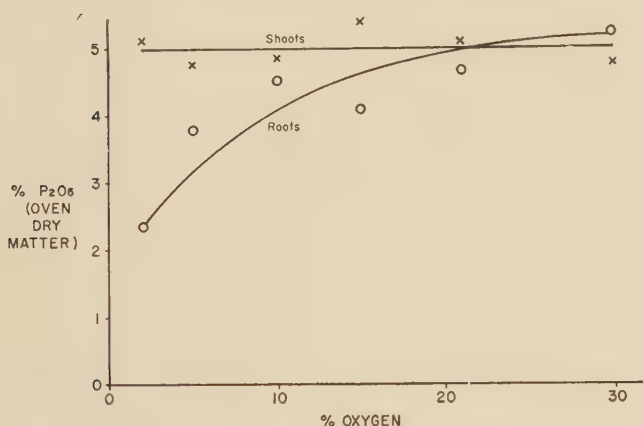


FIGURE 5

The changes in the percentage of phosphorus in the roots and shoots of 20-day-old cocoa seedlings as the oxygen content of the aerating gas is varied. Carbon dioxide held constant at $2\frac{1}{2}$ per cent.

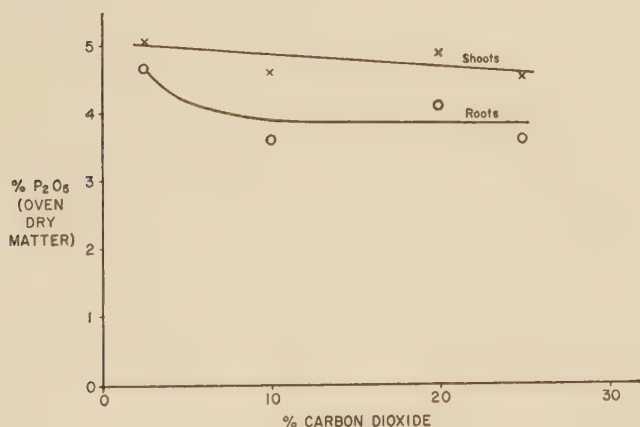


FIGURE 6

The changes in the percentage of phosphorus in the roots and shoots of 20-day-old cocoa seedlings as the carbon dioxide content of the aerating gas is varied. Oxygen held constant at 21 per cent.

Summary

1. The effect of changes in the oxygen and carbon dioxide content of the aerating gas on the growth, and on the nitrogen and phosphorus composition, of young cocoa seedlings grown in nutrient solution has been studied.

2. Optimum root growth as measured by rate of elongation or dry weight increase is obtained with an aerating mixture containing 21% oxygen and $2\frac{1}{2}$ % carbon dioxide. Increasing the oxygen content to 30% results in decreased root growth but shoot growth increases. The dry weight of the whole plant is decreased when the aerating mixture has more than $2\frac{1}{2}$ % carbon dioxide or less than 21% oxygen.

3. The limits for satisfactory root growth may be given as 5% oxygen and 10% carbon dioxide if elongation rate of the tap root is taken as a criterion. On a dry weight basis the lower limit for oxygen must be raised to at least 10%. It should be noted that these limits assume that either oxygen or carbon dioxide is present at its optimal concentration. Compared with other plants, cocoa does not seem particularly intolerant of bad aeration conditions.

4. Shoot nitrogen and phosphorus are not affected by changes in the aerating gas composition. Root nitrogen is only affected at the lowest oxygen and highest carbon dioxide concentration used. Root phosphorus, however, shows a marked dependence on the oxygen concentration in the rooting medium.

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Manurial and cultural experiments on cacao. Part VIII

The effects of fertilisers, shade and spacing on flowering, fruit set and cherelle wilt, 1954-1957, at River Estate, Trinidad

by

G. K. Maliphant

IN an early paper in this series (Havord, Maliphant and Cope, 1955) the results from the first nine months of a programme of flower counting, cherelle tagging and crop reaping under various conditions of shade, spacing and fertilisers were reported. This paper extends these results through the next three crops. Fig. 1 shows the average monthly yields, in mean pods per tree, for the period January, 1954, to September, 1957, and also the cropping periods associated with the above four crops, A, B, C and D.

The methods used in this study have been discussed

in the four cropping periods studied and mean pods per tree for the entire crop.

In interpreting the subsequent results this variation must be remembered, since it explains some of the apparent discrepancies between effects shown on the whole plot and effects shown on the four trees under observation.

The mean annual yields for these four crops were:—

A. 1953-1954	409 lb. dry cacao per acre
B. 1954-1955	1,028 lb. " " "
C. 1955-1956	1,022 lb. " " "
D. 1956-1957	1,198 lb. " " "

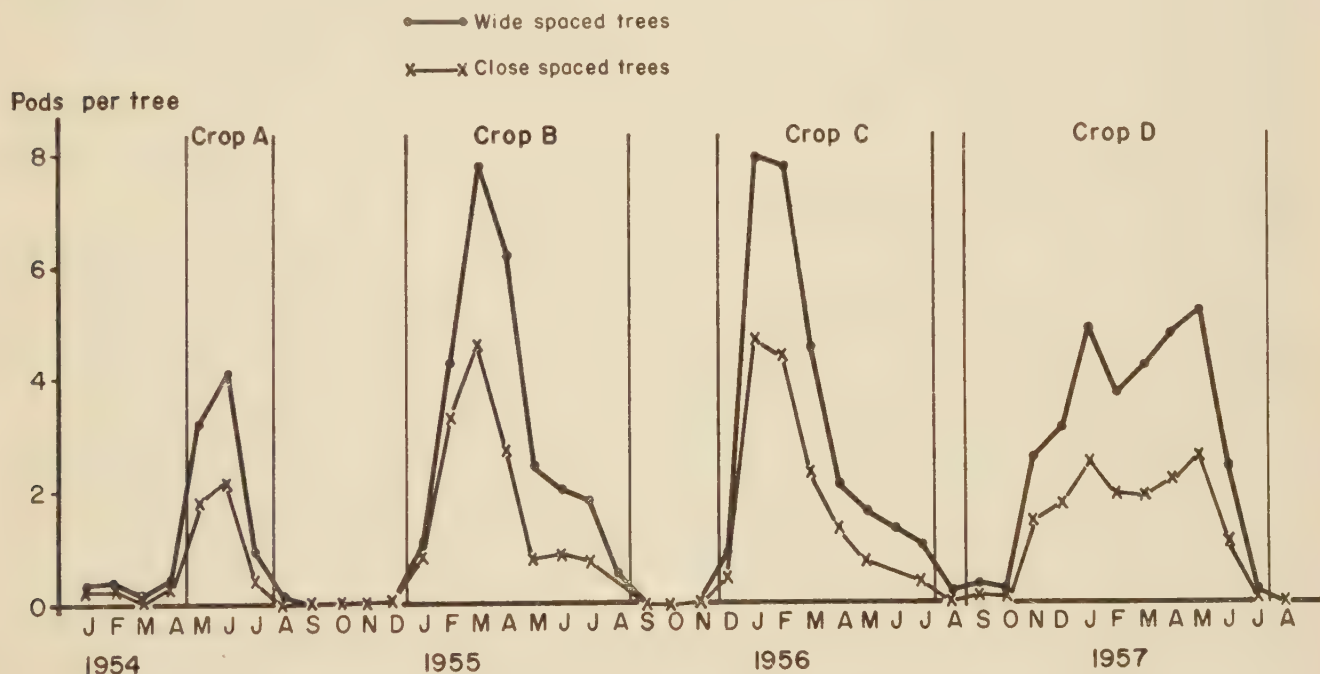


FIGURE 1

Monthly yields (pods per tree) from close-spaced (8 × 8 ft.) and wide-spaced (12 × 12 ft.) cacao—1954-1957.

earlier and it will be noted that only portions of four trees in each plot were studied. An analysis of the variation of the ratio of pods recorded from parts of four trees during the four crop periods to the mean pods per tree from the entire plot in the complete crop year showed that there was a significant difference between the mean ratios from the plots under the four combinations of shade and nitrogen fertiliser (Table I). However, with one exception, significant correlations were found between pods recorded

Thus the three crops under consideration (B, C and D) in this paper are ones where the mean yields were over twice that of the first crop. Since 1957 the mean annual crop has slowly risen to 1,312 lb. dry cacao per acre in 1959-1960.

Details of rates of fertiliser applications and subsequent yields for the period 1954-1958 have already been presented (Maliphant, 1959).

TABLE I

Effect of shade and nitrogen fertiliser on the ratio of pods per tree for the 4 cropping periods studied and mean pods per tree for the entire crop, and the correlation coefficient between pods per tree recorded in the crop-logging programme and mean pods per tree for the entire crop. (n=16 in each case)

		No shade		Shade	
		-N	+N	-N	+N
Crop A					
Ratio	...	.64	.46	.63	.62
Correlation coefficient	...	.93	.86	.66	.67
Crop B					
Ratio	...	.47	.44	.38	.35
Correlation coefficient	...	.83	.93	.34N.S.	.73
Crop C					
Ratio	...	.49	.35	.38	.34
Correlation coefficient	...	.66	.73	.64	.88
Crop D					
Ratio	...	.78	.63	.65	.54
Correlation coefficient	...	.90	.91	.79	.90

N.S.—Non-significant.

The results presented below were obtained from observations during the following periods:—

Crop B

Flowering and cherelle set Aug., 1954-March, 1955

Cherelle wilt Sept., 1954-April, 1955

Pods harvested Jan., 1955-Aug., 1955

Crop C

Flowering and cherelle set July, 1955-Jan., 1956

Cherelle wilt Aug., 1955-Feb., 1956

Pods harvested Dec., 1955-June, 1956

Crop D

Flowering and cherelle set April, 1956-Feb., 1957

Cherelle wilt May, 1956-March, 1957

Pods harvested Sept., 1956-July, 1957

As the observations were made at fortnightly intervals and cacao flowers are very short-lived the flower counts were only a small percentage of the total flower production and hence the data quoted as "cherelle set as percentage of flower count" was not the true percentage set, which may be as low as 1% (Murray, 1953), but was assumed to be proportional to that figure. Cherelles are susceptible to wilt for over two months (Murray, 1953) but it was found in this work that the majority of cherelles which eventually wilted did so before two months. Over the extended periods of time considered the final yield was found to be significantly correlated with the cherelles set five and a half months previously and the cherelles that wilted four and a half months previous to the harvesting.

Shade

In the first crop, as previously reported, shade increased yields. However, in the later crops the reverse was true (Table II) and this resulted from reductions in flowers borne and cherelles set and an increase in percentage wilt. Although flowering and subsequent cherelle set increased markedly with successive crops the proportion of flowers which set fruit fell to less than one-half in crop D and was consistently higher in shaded plots.

Spacing

Wide-spaced trees very highly significantly out-yielded close-spaced trees in all the crops under discussion. As was found in the results from the first crop, this effect is traceable to enhanced flowering and an increase in cherelles set. Cherelle wilt was also higher in close-spaced plots under shaded conditions (Table II).

Nitrogen fertiliser

Nitrogen fertiliser cause a marked increase in yield in the absence of shade. This was largely due to enhanced flowering and subsequent cherelle set despite a reduction in the percentage of flowers which set fruit (Table II). Cherelle wilt was reduced in wide-spaced plots but there was no effect in the later two crops in close-spaced plots. In the previous crop (Havord, Maliphant and Cope, 1955) nitrogen fertiliser increased the percentage wilt in close-spaced plots.

In the presence of shade (Table III) nitrogen fertiliser had little effect on the first two crops under consideration but caused a reduction in yield in the third crop due largely to reduced flowering and cherelle set, particularly in the wide-spaced plots. In close-spaced plots an increase in percentage wilt was also partly responsible for the loss in crop.

Phosphate fertiliser

Yield responses to phosphate fertiliser were much lower than responses to nitrogen fertiliser in the absence of shade. However, small responses were obtained in shaded plots particularly in wide-spaced plots (Table III). Phosphate fertiliser generally enhanced flowering, but in shaded plots reduced the percentage of flowers which set cherelles. There were no consistent effects of phosphate fertiliser on cherelle wilt.

Potash fertiliser

The effects of potash fertiliser noted on the first crop (Havord, Maliphant and Cope, 1955) have changed with successive crops (Table IV). Potash fertiliser caused an increase in flowering but tended to reduce the percentage of flowers setting, particularly in unshaded plots and, in Crop B, in shaded plots.

In unshaded plots in Crops B and C the above effects, with an increase in cherelle wilt due to potash fertiliser, resulted in an overall depression in yield. In Crop D potash fertiliser caused a slight drop in cherelle wilt and the resulting effect on crop reaped was nil.

In shaded plots in Crop B the very high percentage of flowers which set cherelles in the absence of potash fertiliser resulted in a reduction of yield due to potash fertiliser. In the two later crops, despite higher percentage wilt, the enhanced flowering in potash fertilised plots has resulted in an overall increase in yield.

Discussion

When the results reported above are compared with those reported for the first crop it will be noted that many of the effects vary from crop to crop. It is probable, therefore, that factors outside the experiments are having a marked effect on the stages of crop production and are strongly interacting with the fertilisers, shade and spacing. Although that it is probably true under normal conditions flower production is never limiting to the final crop

(Murray, 1953), and that percentage cherelle wilt is the main controlling factor, the results presented show that crop responses to fertilisers are more the result of increased flower production and fruit set than on reduced cherelle wilt.

The beneficial effect of potash fertiliser on cherelle wilt found by Cope (1939) and Humphries (1943) was not confirmed in the results presented. These earlier workers used cacao trees much older than those used for this work and it is possible that the beneficial effect of potash fertiliser will become apparent as the cacao trees mature. As has already been demonstrated (Maliphant, 1959), the effect of potash fertiliser on final yield has increased with age.

Summary

Results of observations on flowering, cherelle set, wilt and crop reaped for three successive crops (1955-1957) are presented, and compared with the 1954-1955 results previously reported. Flowering increased markedly with successive crops, but the percentage of flowers which set fruit, which rose from the 1954-1955 crop to the 1955-1956 crop, fell sharply in successive crops.

Flowering and cherelle set were lower in shaded plots than in unshaded plots. However, the resultant of these effects was partially offset by an increased percentage of flowers which set cherelles, but cherelle wilt was higher in shaded plots than in unshaded plots.

Flowering was also much reduced in close-spaced plots, especially in the absence of shade, and cherelle wilt was

higher in close-spaced plots, especially in the presence of shade.

Nitrogen fertiliser increased flowering, particularly in the absence of shade, and decreased cherelle wilt in wide-spaced plots.

Phosphate fertiliser increased flowering and tended to reduce cherelle wilt in the absence of shade, resulting in a general small positive increase in yield.

Potash fertiliser increased flowering and cherelle wilt but reduced the percentage of flowers which set cherelles. These effects resulted in yield increases only in shaded plots.

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TABLE II

Effect of shade, spacing and nitrogen fertiliser on flowering, cherelle set, percentage wilt and pods reaped for 3 successive crops (B, C and D) (totals from 4 trees)

				Flower count			Cherelle set			Cherelles set as % of flower count			Percentage wilt			Pods reaped		
Unshaded	Wide spaced	-N +N		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				348	616	1,164	118	190	267	41.7	33.0	23.3	74	75	67	31	48	88
				621	956	1,912	214	198	340	38.3	22.5	18.3	66	72	66	71	57	116
Means				484	786	1,538	166	194	303	40.0	27.7	20.8	70	73	67	51	52	102
	Close spaced	-N +N		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				214	441	633	97	111	136	46.5	28.1	21.7	69	71	66	30	32	46
				286	566	875	107	106	143	40.3	20.3	16.9	75	71	66	27	31	48
Means				250	503	754	102	108	140	43.4	24.2	19.3	72	71	66	28	32	47
MEANS	...	...	...	367	645	1,146	134	151	221	41.7	26.0	20.0	70	71	67	39	42	75
Shaded	Wide spaced	-N +N		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				214	493	1,289	145	192	294	66.1	40.9	23.1	76	80	70	36	39	87
				205	519	952	119	160	232	63.2	38.4	26.3	70	76	68	36	39	75
Means				210	506	1,120	132	176	263	64.7	39.6	24.8	73	76	69	36	39	81
	Close spaced	-N +N		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				247	341	672	124	99	147	55.9	30.5	22.5	80	82	76	25	21	36
				236	308	619	115	93	130	65.0	33.1	21.3	81	81	81	23	19	26
Means				241	324	645	120	96	138	60.4	31.8	21.9	81	81	78	24	20	31
MEANS	...	...	...	226	415	882	126	136	200	62.6	35.7	23.3	77	79	73	30	30	56

TABLE III

Effect of shade, spacing and phosphate fertiliser on flowering, cherelle set and wilt and pods reaped for 3 successive crops (B, C and D) (totals from 4 trees)

				Flower count			Cherelle set			Cherelle set as % of flower count			Percentage wilt			Pods reaped		
Unshaded	Wide spaced	-P +P		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				562	728	1,423	180	178	290	32.0	24.5	20.4	73	74	66	49	46	99
				406	844	1,653	151	210	317	37.2	24.9	19.2	65	72	67	53	58	104
	Close spaced	-P +P		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				246	471	650	98	102	117	39.8	21.7	81.0	74	72	65	26	29	41
				253	536	858	105	114	162	41.5	21.3	18.9	71	71	67	32	33	54
Shaded	Wide spaced	-P +P		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				194	390	1,021	123	161	241	62.4	41.3	24.3	71	77	70	35	37	74
				226	623	1,220	141	192	278	62.4	30.8	22.8	74	78	69	37	42	87
	Close spaced	-P +P		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				181	289	606	106	94	133	58.6	32.5	21.9	78	83	77	23	18	29
				301	360	685	133	99	145	44.2	27.5	21.2	82	78	78	23	21	31

TABLE IV

Effect of shade and potash fertiliser on flowering cherelle set and wilt and pods reaped from 3 successive crops (B, C and D) (totals from 4 trees)

				Flower count			Cherelle set			Cherelle set as % of flower count			Percentage wilt			Pods reaped		
Unshaded		-K +K		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				356	593	1,081	138	152	225	38.7	25.6	21.6	69	70	67	42	48	75
				378	696	1,251	131	151	219	34.7	21.6	17.8	71	76	66	38	36	75
Shaded		-K +K		B	C	D	B	C	D	B	C	D	B	C	D	B	C	D
				193	350	828	130	117	187	67.6	33.6	22.5	76	77	71	31	27	54
				258	481	938	120	155	215	46.6	32.2	22.9	77	80	73	28	32	58

The nutrition of cacao. Part III

Bark nitrogen and phosphorus levels

by
G. K. Maliphant

IN a previous publication (Jones, Maliphant and Havord, 1957) details of a crop-logging procedure for cacao were given. The present paper contains some of the results of bark analyses on samples taken during the period May, 1956, to July, 1957. Four trees were selected at random from each of the 64 main plots in Experiment I, River Estate, Trinidad (Havord, 1953), and bark samples taken from each tree at 4 ft. from the ground and these samples bulked. Four weeks later a different random selection of four trees was sampled. This process was repeated 17 times during this period. The cacao was ICS 1, planted in 1949 on a sandy loam soil of imperfect drainage. The samples after drying and grinding were chemically analysed according to the procedures previously described (Jones, Maliphant and Havord, 1957).

The present paper gives results of nitrogen and phosphorus estimations in the 17 sets of samples taken.

Bark nitrogen content (N% ODM)

Shade, spacing and nitrogen fertilisers affected bark nitrogen contents. The spacing effect was relatively constant throughout the year, bark samples from close-spaced trees containing 0.02% less nitrogen than samples from wide-spaced plots. This effect was significant in June-August, 1956, December, 1956, January, May and June, 1957.

Shade and nitrogen fertiliser had much larger effects on bark nitrogen than spacing (Fig. 1). Shade increased bark nitrogen significantly throughout the year, the effect being larger in the absence of nitrogen fertiliser.

Nitrogen fertiliser caused significant increases in bark nitrogen throughout most of the year in the absence of shade, but not in its presence. The magnitude of these effects does not appear to be associated with the fertiliser applications in May, 1956 and 1957.

It is apparent from Fig. 1 that the fertiliser and shade effects do not alter the general pattern of seasonal changes in bark nitrogen. In Fig. 2 the mean bark nitrogen content for the 64 plots in the experiment is plotted against the time of sampling. The upper graph in Fig. 2 is the mean soil moisture content (% v/v, plotted on an inverted scale) in the top 3 in. of soil six weeks prior to bark sampling; these data were derived from weekly soil moisture measurements taken as part of the crop-logging procedure (Jones, Maliphant and Havord, 1957). There is an obvious association between soil moisture and subsequent levels of bark nitrogen. This is probably due to the fact that a dry spell of weather causes an increase in soil nitrate-nitrogen. It is the metabolism of this nitrate, readily absorbed by the plant during a subsequent wetter period, which has caused

a sharp rise in bark nitrogen in May, 1957. The methods used for chemical analysis of the bark did not estimate nitrate-nitrogen in the bark but nitrogen bound as ammonium ions or in organic compounds, *e.g.* amines and protein.

Bark phosphorus content (P₂O₅ % ODM)

Shade and nitrogen fertiliser had profound negative effects on the levels of bark phosphorus throughout the period of sampling (Fig. 3).

Despite the lack of precision in estimating the shade effect statistically this effect was significant at most of the sampling dates. The effect of nitrogen fertiliser was of the same order of magnitude as that of shade, and the shade x nitrogen fertiliser interaction was positive. The resultant of these effects was that there were no significant differences between phosphorus levels in (1) shaded, nitrogen fertilised, (2) shaded and (3) nitrogen fertilised plots, but these levels were very significantly lower than in samples from unshaded plots not receiving nitrogen fertiliser. At the same time both phosphate and potash fertilisers significantly affected bark phosphorus levels in the absence of shade and nitrogen fertiliser (Table I) but not in their presence.

TABLE I

Effect of phosphate and potash fertilisers on bark phosphorus content (P₂O₅ % ODM) in the absence of nitrogen fertiliser and permanent shade

Fertiliser	—	P	K	PK	L.S.D. (P=0.05, n=28)
May (i), 1956...	.311	.453	.491	.606	.112
May (ii) ...	.419	.460	.385	.593	.096
June ...	.451	.531	.539	.545	.094
July ...	.463	.613	.552	.606	.098
August ...	.478	.389	.524	.535	.106
September ...	.380	.454	.452	.459	.135
October ...	.390	.528	.440	.517	.092
November ...	.372	.577	.460	.579	.066
December ...	.391	.444	.446	.497	.084
January, 1957...	.347	.487	.501	.557	.098
February ...	.360	.539	.476	.664	.070
March... ..	.467	.670	.470	.632	.070
April ...	.399	.527	.502	.600	.152
May (i) ...	.416	.526	.585	.649	.098
May (ii) ...	.520	.679	.532	.701	.116
June ...	.407	.636	.562	.712	.080
July ...	.410	.727	.649	.756	.135

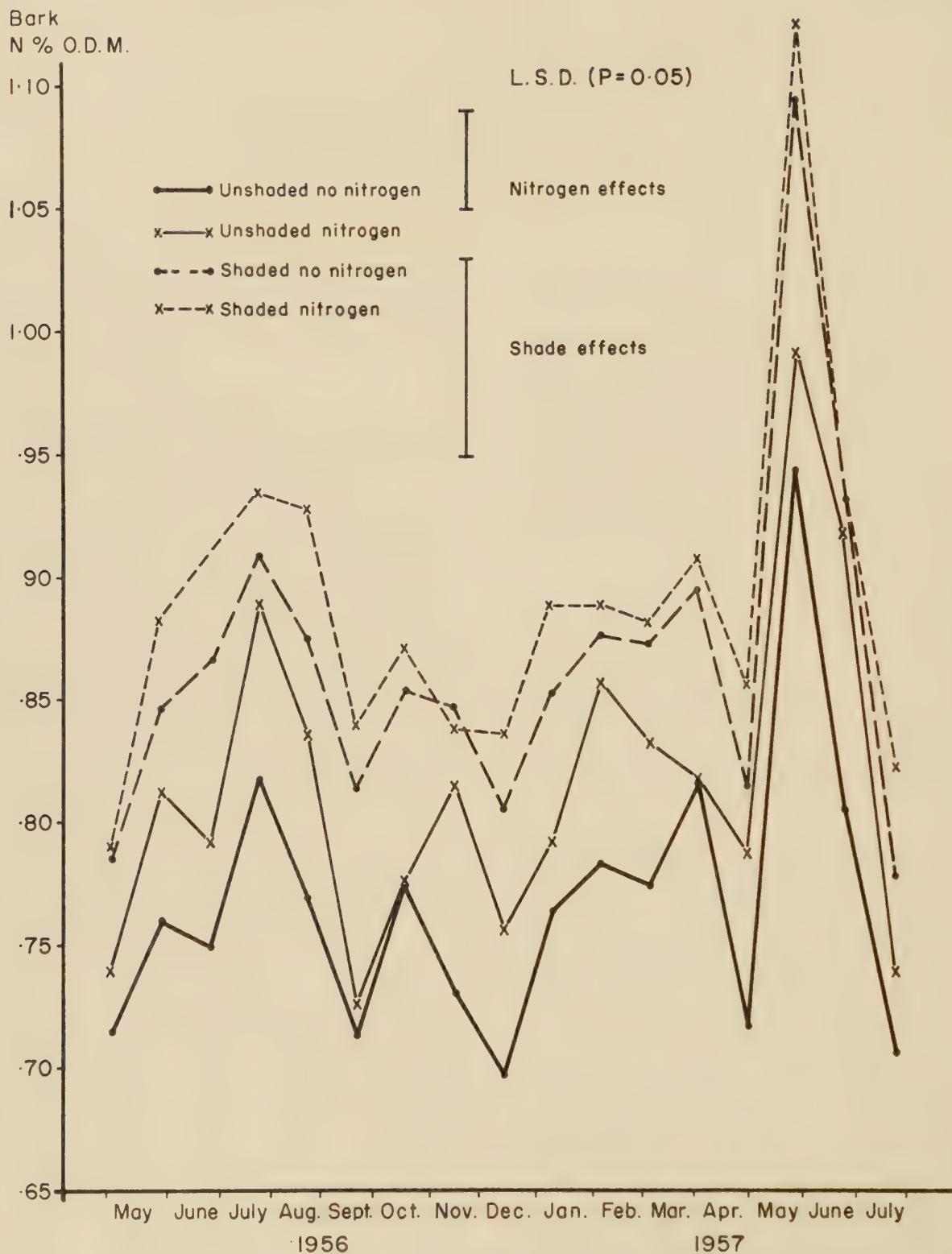


FIGURE 1
Effect of time of sampling, shade and nitrogen fertiliser on bark nitrogen (N % ODM).

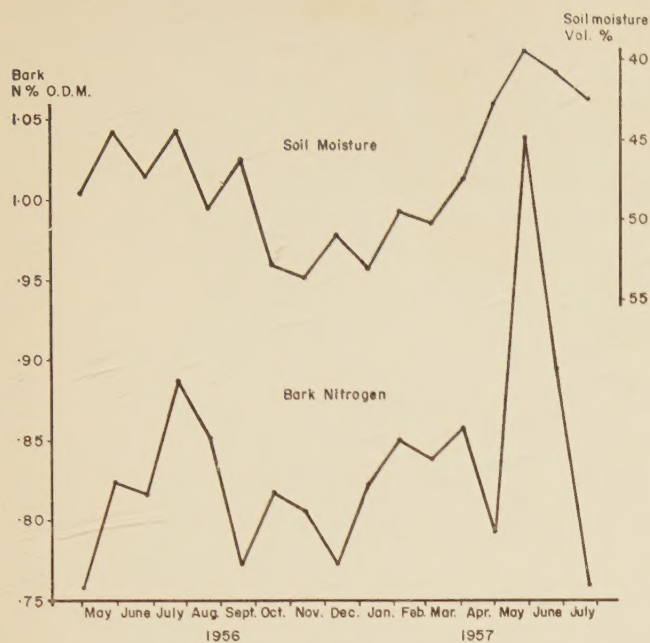


FIGURE 2

Seasonal changes in bark nitrogen (N % O.D.M.) and their relationship with soil moisture six weeks previous.



FIGURE 3

Seasonal changes in bark phosphorus (P₂O₅ % O.D.M.) and the effect of shade and/or nitrogen fertiliser.

It will be noted that shade and nitrogen fertiliser reduced bark phosphorus levels by 40%, whereas in the absence of shade and nitrogen fertiliser, phosphate and potash fertilisers raised bark phosphorus levels by as much as 80%.

Shade and nitrogen fertiliser caused a reduction, and phosphate fertiliser caused an increase, in leaf phosphorus levels (Malipant, 1959) but the changes were generally less than 10% of the mean.

Potash fertiliser had no marked effect on leaf phosphorus levels but the effect was large on bark phosphorus levels, particularly in the absence of phosphate fertiliser.

Bark content and yield

Bark contents of nitrogen and phosphorus and the N/P₂O₅ ratio were examined for correlation with yield (pods per 4 trees, 1956-7) from the four trees sampled. There were no significant correlations between bark nitrogen levels and yield. Significant correlations between bark phosphorus levels and yield were obtained only in samples taken from unshaded wide-spaced plots. The correlation coefficient varied from -0.47 (May, 1957) to -0.80 (March, 1957) (n=16 in each case). There were no significant correlations between yield and bark N/P₂O₅ ratio in shaded plots, and poor correlations were found in close-spaced plots. Significant correlations were obtained between yield and bark N/P₂O₅ ratio in unshaded wide-spaced plots but they were no higher than those obtained for yield-bark P₂O₅ correlations.

Discussion

Very little work has been published on the results of bark analysis of perennial crops. Bolle Jones (1957) found significant latex yield correlations with bark phosphorus levels in rubber. The levels of nitrogen and phosphorus in cacao bark shown above were considerably higher than in rubber and slightly higher than in apples (Mason and Whitfield, 1960). Cameron *et al* (1954) found nitrogen levels in Valencia orange bark higher than those shown above for cacao, but phosphorus levels were considerably lower. None of these workers, however, studied the effects of fertilisers on bark constituents.

The variations in nitrogen and phosphorus content of bark of mature shaded cacao has been studied by Humphries (1950) who found a seasonal change in bark nitrogen and phosphorus levels, but, despite a statement that bark phosphorus was not correlated with yield, later showed a significant negative correlation between these factors. When the results from shaded trees presented above are converted to the same basis as Humphries (gm. of the element per 100 gm. of water) it is found that nitrogen levels were much higher in the mature trees of Humphries but fluctuations were less. In the case of phosphorus content, the younger trees used in the work reported above had higher levels and much greater fluctuations than mature trees, and a different pattern of seasonal change.

There appears to be little relationship between the effects of fertilisers on bark constituents and applications of fertilisers, which were in May of each year. This may be due to the fact that samples were not taken within a few days of fertiliser application. It has been found (Moss, private communication) that an abundance of available potassium in the rooting zone of cacao from fertiliser applications is only present in River Estate soil for a few days subsequent to such applications. Presumably nitrogen available in ammonium sulphate fertiliser disappears from the rooting zone equally fast.

Summary

Results of analyses of cacao bark samples taken at four weekly intervals May, 1956-July, 1957, are presented.

An apparent association between bark nitrogen and soil moisture content six weeks earlier is shown.

The profound effect of shade and nitrogen fertiliser on bark phosphorus content is demonstrated and a larger effect of phosphate and potash fertilisers in the absence of shade and nitrogen fertiliser described.

It is suggested that the absence of a rise in bark nitrogen immediately after fertiliser applications is associated with the fact that the effect of fertilisers, on River Estate soil, of increasing available nitrogen and potassium is transitory.

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